1997-1999 REGION 3, U. S. FISH AND WILDLIFE SERVICE SECTION 6 GRANT FINAL REPORT.

Project Title: Determination of basic reproductive characteristics of the winged mapleleaf mussel (*Quadrula fragosa*) relevant to recovery. MWBC 2601400

Project Number: E-1-27

Submitted to: *U. S. Fish and Wildlife Service*

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Job Number 2: Host Fish Determinations.

See enclosed report entitled: “Brooding Behavior and Suitable Host for the Winged Mapleleaf (*Quadrula fragosa*” by Mark C. Hove, Jennifer E.

Job Number 3: Glochidial Identifications.

See enclosed report entitled: “Examination The Larval Stage (Glochidia) Of The Winged Mapleleaf Mussel (Quadrula fragosa” by Daniel J. Hornbach, Jesse Kroese and Benjamin Miller.

JOB REPORT SUMMARIES

Job Number 1: Gravidity Period Determination

The purpose of the investigation was to determine the brooding period of the federally endangered freshwater mussel *Quadrula fragosa* (Conrad, 1835) (Mollusca: Bivalvia: Unionidae) in the St. Croix River, Minnesota and Wisconsin. Approximately every two weeks, we examined the brooding condition of ten amblemine species during the open-water seasons of 1997-1999. These species included *Quadrula metanevra* (Rafinesque, 1820), *Q. quadrula* (Rafinesque, 1820), *Q. p. pustulosa* (I. Lea, 1831), *Q. fragosa* (Conrad, 1835), *Tritogonia verrucosa* (Rafinesque, 1820), *Cyclonaias tuberculata* (Rafinesque, 1820), *Elliptio dilatata* (Rafinesque, 1820), *Fusconaia flava* (Rafinesque, 1820), *Pleurobema sintoxia* (Rafinesque, 1820) and *Amblema p. plicata* (Say, 1817).

Four temporal subsets of brooders were found. The first, very early brooders, included *Q. metanevra*, *Q. p. pustulosa*, *T. verrucosa* and *C. tuberculata.* These brooded from April 21 to July 29. The early subset, which included *E. dilatata*, *F. flava* and *P. sintoxia,* brooded from May 4 to Aug. 26. The mid-season subset included a single species: *Amblema p. plicata*. It brooded from June 10 to Aug. 5; about half as long as the previous two subsets. The final subset, late season brooder, included only one species: *Q. fragosa*. It had a very short 5.8-week brooding period extending from Aug. 31 to Oct. 11. Brooding duration was less than 75% of other species. Brooding periods for all species from all four subsets overlapped except for *Q. fragosa.* This species had a distinct, late and short brooding period that did not overlap with any other amblemine. Its brooding period did not overlap with brooding period literature records of the closely related *Q. quadrula* suggesting species goodness.

The frequency of brooding during the brooding period of *Q. fragosa* was correlated with downstream water temperatures. During 1998, 21.3% were found brooding but during other years only 2.4% brooded. The mean annual temperature was about 12% warmer in 1998 than the four-year mean and was warmer than any other year from 1996-1999.

Growth rates of Q. fragosa, measured as total length, decreases as age increases. One year-old individuals grew about 8mm annually while 22 year-olds showed no measurable growth. The youngest gravid individual found was 8 years old while the smallest was 65 mm total length.

Job Number 2: Host Fish Determinations.

The winged mapleleaf (*Quadrula fragosa* (Conrad, 1835)) historically occurred in rivers across eleven U.S. states but now only occurs in a small portion of a few rivers. The glochidial host(s) for this federally endangered species are unknown which makes it nearly impossible to determine the viability of imperiled mussel populations either in degraded habitats, where they now occur, or in habitats being considered for relocation of mussels. We studied brooding winged mapleleaf in the St. Croix River and at the Wet Laboratory, University of Minnesota. During the brooding period a swollen excurrent aperture was observed among brooding and some non-brooding individuals. Glochidia were released individually or in conglutinates. Suitable glochidial hosts were determined using a standard artificial infestation protocol. Forty-five trials were conducted on thirty-five fish species or mudpuppies. Two juvenile winged mapleleaf were collected from a single channel catfish. Juvenile mussels grew substantially during the encystment period. We attempted to collect winged mapleleaf juveniles from naturally infested fishes but none of the recovered mussels were winged mapleleaf. Additional work is needed to determine the function of the swollen excurrent aperture displayed during the brooding season, and to verify that Ictalurids serve as glochidial hosts under laboratory and natural conditions.

Job Number 3: Glochidial Identifications.

The goal of this study was to develop a method to identify the glochidia (larval stage) of the endangered winged mapleleaf mussel (*Quadrula fragosa*). This species of mussel is currently found only in the St. Croix River and little is known of its basic biology. Freshwater mussels utilize a fish host for dispersal of the larval stage and the species of fish which are suitable hosts for *Q. fragosa* are unknown.

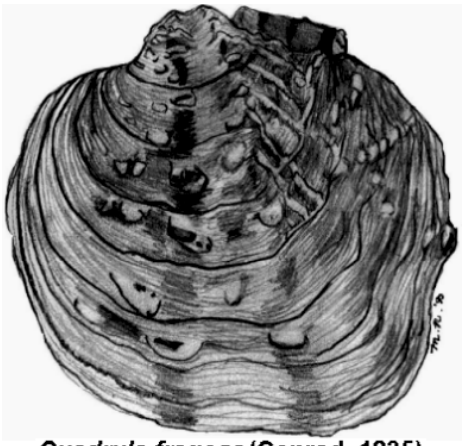
We explored 2 methods to differentiate the glochidia of *Q. fragosa* from other species of unionids in the St. Croix River. One method involved collecting glochidia from species of *Quadrula* and taking morphometric measurements on specimens under a scanning electron microscope (SEM). The other method involved using DNA fingerprinting using the polymerase chain reaction (PCR). We examined the ITS-1 region of the genome and subjected amplified DNA to a variety of restriction enzymes.

There were differences in the sizes of the glochidia examined for 3 species of *Quadrula*. We obtained glochidia from one Quadrula *fragosa* collected in September 1998. These glochidia were smaller than those obtained from *Q. pustulosa* or *Q. metanevra*. While this method holds promise for identification of *Q. fragosa* glochidia more data is required.

We were able to differentiate *Q. fragosa* from the other 16 species of mussels examined from the St. Croix River. This differentiation could be done by using a single restriction enzyme (MSP1). We were able to obtain sufficient DNA from a small number of glochidia to carry out the PCR reaction. This method provides a simple diagnostic for *Q. fragosa* glochidia.

DETERMINATION OF BASIC REPRODUCTIVE CHARACTERISTICS OF THE WINGED MAPLELEAF MUSSEL (*QUADRULA FRAGOSA*) RELEVANT TO RECOVERY.

JOB 1: DETERMINATION OF GRAVIDITY PERIOD.



Quadrula fragosa **(Conrad, 1835)**

Final Report

October, 2000.

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SUMMARY

The purpose of the investigation was to determine the brooding period of the federally endangered freshwater mussel *Quadrula fragosa* (Conrad, 1835) (Mollusca: Bivalvia: Unionidae) in the St. Croix River, Minnesota and Wisconsin. Approximately every two weeks, we examined the brooding condition of ten amblemine species during the open-water seasons of 1997-1999. These species included *Quadrula metanevra* (Rafinesque, 1820), *Q. quadrula* (Rafinesque, 1820), *Q. p. pustulosa* (I. Lea, 1831), *Q. fragosa* (Conrad, 1835), *Tritogonia verrucosa* (Rafinesque, 1820), *Cyclonaias tuberculata* (Rafinesque, 1820), *Elliptio dilatata* (Rafinesque, 1820), *Fusconaia flava* (Rafinesque, 1820), *Pleurobema sintoxia* (Rafinesque, 1820) and *Amblema p. plicata* (Say, 1817).

Four temporal subsets of brooders were found. The first, very early brooders, included *Q. metanevra*, *Q. p. pustulosa*, *T. verrucosa* and *C. tuberculata.* These brooded from April 21 to July 29. The early subset, which included *E. dilatata*, *F. flava* and *P. sintoxia,* brooded from May 4 to Aug. 26. The mid-season subset included a single species: *Amblema p. plicata*. It brooded from June 10 to Aug. 5; about half as long as the previous two subsets. The final subset, late season brooder, included only one species: *Q. fragosa*. It had a very short 5.8-week brooding period extending from Aug. 31 to Oct. 11. Brooding duration was less than 75% of other species. Brooding periods for all species from all four subsets overlapped except for *Q. fragosa.* This species had a distinct, late and short brooding period that did not overlap with any other amblemine. Its brooding period did not overlap with brooding period literature records of the closely related *Q. quadrula* suggesting species goodness.

The frequency of brooding during the brooding period of *Q. fragosa* was correlated with downstream water temperatures. During 1998, 21.3% were found brooding but during other years only 2.4% brooded. The mean annual temperature was about 12% warmer in 1998 than the four-year mean and was warmer than any other year from 1996-1999.

Growth rates of Q. fragosa, measured as total length, decreases as age increases. One year-old individuals grew about 8mm annually while 22 year-olds showed no measurable growth. The youngest gravid individual found was 8 years old while the smallest was 65 mm total length.

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Introduction

Prior to about 1925, the winged mapleleaf freshwater mussel, *Quadrula fragosa* (Conrad, 1835) (Mollusca: Bivalvia: Unionidae), occurred in at least 11 states and about 35 rivers (U. S. Fish and Wildlife Service, 1991; Posey, W. R., J. L. Harris, and G. L. Harp, 1996). At present, it remains in three streams at most, and its loss has been confirmed in nearly all others. One of the three remaining populations is located in the St. Croix River bordering the states of Minnesota and Wisconsin. This loss of historic geographic range led the U. S. Fish and Wildlife Service to list the winged mapleleaf freshwater mussel as federally endangered in 1991.

At the time of federal listing, very little biological information about the species existed. Longevity, host fishes, brooding period, growth rate, associated microhabitat, population characteristics and breeding behavior were unknown. This lack of essential information limited the number of reasonably effective recovery actions available.

A Federal Recovery Plan, completed in 1997 (U. S. Fish and Wildlife Service, 1997), identified a number of threats to the species and outlined suggested species recovery actions. Among the suggested recovery actions, addressing the threat of a potential zebra mussel (*Dreissena polymorpha (Pallas, 1771)*) invasion and preservation of the St. Croix River population was viewed as essential to preventing extinction. The plan also called for the establishment of additional secure, persistent populations in order to reclassify and de-list the species.

The U. S. Fish and Wildlife Service (USFWS) recognized that population preservation, establishment and augmentation require a basic understanding of this mussel's biology. Therefore, they outlined essential recovery actions aimed at understanding reproductive biology, reproductive phenology and reproductive parameters.

This report addresses some of these essential recovery actions related to reproduction. Specifically, the principle objective of this investigation is to discover and document the reproductive period of the winged mapleleaf freshwater mussel in the St. Croix River. Secondary objectives include documentation of winged mapleleaf growth and the brooding and glochidial release period of other members of the subfamily ambleminae.

Description of Study Site

Field sampling of mussels occurred within a 0.72 km^2 area of the St. Croix River bordering the states of Wisconsin and Minnesota, USA (Figure 1) and is known as the Interstate Parks site. This site, located in Chisago County, Minnesota and Polk County, Wisconsin extends from St. Croix River km 80.7 to 85.0 and is centered around 45º 22' 57"N, 92º 40' 29"W. The drainage area upstream of the

study site is 16161 km^2 (USGS Gaging Station 05340500 Data) and is primarily forested. Riparian zones on the main stem and a major tributary, the Namekagon River, are designated as National Scenic Riverway under the Wild and Scenic Rivers Act of 1968. The mean daily flow at the site for the period of 1910 to 1998 is 122 m^3/s (4333 ft^3/s) and the summer mean depth is approximately 0.9m.

We chose this site because it is presumed to contain the greatest number of winged mapleleaf mussels in the drainage basin (Doolittle, 1988). Total mussel population densities in the upper half of this study site were 16.3/m^2 and population densities of winged mapleleaf were 0.027/m^2 (Wisconsin Department of Natural Resources data). The study site contains a diverse mussel assemblage. A total of 35 of the 42 St. Croix River basin freshwater mussel species occur within the Interstate Parks site.

Methods

We conducted winged mapleleaf field sampling from 7 May through 4 November 1997; 9 April through 23 October 1998; and 27 April through 18 October 1999. We collected samples every two weeks during 1997 and during the spring and summer 1998. During the fall of 1998 and 1999, sampling occurred every week. No sampling occurred during the summer of 1999. We sampled other amblemines during the same time periods except during the fall of 1999.

A total of 38 collection stations where chosen within the study site that we presumed, based on previous experience, to contained relatively higher population densities of winged mapleleaf mussels. These station locations generally contained a gravel or sand and gravel substrate with relatively swift currents.

We used Self Contained Underwater Breathing Apparatus (SCUBA) equipped divers who randomly collected winged mapleleaf mussels and other amblemines from these stations. Divers scoured the streambed by hand often digging up to 10cm. Mussels were sensed tactually and visually. Divers spent relatively more time at locations and in microhabitats where winged mapleleafs had been found.

About 20 non-winged mapleleaf specimens of each of eight species were collected during each of the 39 sampling events. About 10 winged mapleleaf specimens were collected during each 1997 sampling event and about 20 during each event in 1998 and 1999.

Upon finding a winged mapleleaf specimen, divers immediately placed it in a sealed, clear plastic 0.5 to 3.8L bag with river water or brought it to the surface. Other amblemines were placed in a fine-meshed nylon bag. Mussels were brought to the surface and processed. Total length and height were measured to the nearest mm using vernier calipers. Total length was defined as the greatest anterior to posterior distance approximately parallel to the hinge line. Total height was defined as the greatest dorsoventral distance roughly perpendicular to the total length, excluding the umbo. Total age in years was estimated by counting external

growth checks on the shell (annuli). The total length of each discernible winged mapleleaf growth check was measured and its corresponding age recorded.

We examined each mussel for gravidity. An individual was considered gravid if at least one of the four demibranchs (i.e. one of the four halves of the two gills) contained either eggs or glochidia. We did this by gently prying apart the two valves using reverse pliers or a blunt-tipped blade and visually examined the demibranchs for width, transparency and coloration. For some individuals, especially specimens of winged mapleleaf, we slid the end of a Huber Probe beneath a demibranch to ascertain transparency and width. On two initially inconclusive occasions, we pricked a demibranch using a needle to determine gravidity. Other winged mapleleaf mussels, whose gravidity status was inconclusive in the field, were transported to a wet laboratory. These were observed for about 2 weeks for evidence of abortion or release. Mussels that released demibranch contents were considered gravid; those that did not were recorded as not gravid.

In order to minimize stress to winged mapleleaf from gravidity examinations, no winged mapleleaf individuals were examined more than once in a two-week period. Usually, winged mapleleaf individuals were examined only twice in one year and at most twice during their 5 to 6-week brooding period.

All winged mapleleaf mussels collected living had a unique specimen number etched on the exterior of each valve. Most gravid or suspected gravid winged mapleleafs were carefully returned to the streambed by hand within two weeks. All other amblemines were similarly returned to the streambed, near their collection location, within a few hours of collection. All returned mussels were replaced in their natural orientation and partially buried by hand into the substrate. About 80 winged mapleleaf were aggregated at several locations near their original collection location to facilitate recapture.

Beginning and ending points of brooding periods were projected using the following method. For a given year, a geometric midpoint was chosen between the month and day a particular species was first observed gravid and the previous negative sampling date. If available, points similarly derived from the remaining two years were averaged with the first. This one to three-year average was then considered the average projected initial brooding month and day. A similar procedure projected the average brooding end date.

Water temperature data were collected from two sources. During mussel sampling events at the Interstate Parks site, temperatures were taken at depth of 15 cm using a standard stream thermometer. These data were used to determine temperatures associated with mussel brooding condition. The second temperature data source was provided by Xcel Energy, Inc. and is from the cooling system intake structure of the A. S. King Power plant located at river km 35.8 in Bayport, Minnesota. This data was used to compare relative ambient downstream water temperatures among the years 1997 to 1999.

Results and Discussion

Amblemine Gravidity Periods

Nine of the ten amblemine species examined were found gravid including the federally endangered winged mapleleaf. Brooding periods and temperatures for these species are given in Appendix A. The actual observed date ranges of brooding by year are given below. Very few specimens of *Q. quadrula* were found and these data are not included in the table below.

Table 1. Actual Amblemine Brooding Dates, 1997-1999.

Species	First Observed	Last Observed
Quadrula metanevra	05/07/1997	07/29/1997
	04/28/1998	08/03/1998
	04/27/1999	NA
Q. p. pustulosa	05/07/1997	07/16/1997
	04/28/1998	07/06/1998
Tritogonia verrucosa	05/07/1997	06/03/1997
	04/28/1998	06/08/1998
	04/27/1999	NA
Cyclonaias tuberculata	05/07/1997	07/29/1997
	04/28/1998	07/06/1998
Elliptio dilatata	05/20/1997	07/29/1997
	05/11/1998	07/20/1998
Fusconaia flava	06/03/1997	08/12/1997
	05/11/1998	08/03/1998
Pleurobema sintoxia	05/20/1997	07/29/1997
	05/11/1998	08/03/1998
Amblema p. plicata	06/16/1997	07/16/1997
	06/08/1998	07/20/1998
Q. fragosa	09/24/1997	09/24/1997
	09/10/1998	10/08/1998
	09/21/1999	09/21/1999

Four subsets of brooders, based on initialization and length of brooding period, were found. The first set, very early brooders, included *Q. metanevra*, *Q. p. pustulosa*, *Tritogonia verrucosa* and *Cyclonaias tuberculata* (Figure 2). These began brooding during the 16th week of the year (April 21) and generally extended 14 weeks to the 30th week of the year (July 29). Brooding began when rising spring water temperatures reached 10°C and ended 1 week prior to the start of late

summer cooling (25°C). The brooding duration of *T. verrucosa* was anomalous for this subset. It brooded only 8 weeks terminating brooding by the 24th week (June 24) while early summer water temperatures were about 23°C and still rising.

The second subset, early brooders, included *Elliptio dilatata*, *Fusconaia flava*, and *Pleurobema sintoxia*. These were initially found gravid during the 19th week (May 14) and brooded for about 15 weeks until about the 34th week (Aug. 26). Brooding began when rising spring water temperatures reached 16°C and ended 3 weeks after the start of late summer cooling (22°C). Compared to the very early brooders, early brooders initialized 3 weeks later and ended 4 weeks later; both brooded about the same length of time.

The third subset, mid-season brooder, included a single species: *Amblema p. plicata*. It had a relatively short 8-week brooding period extending from the 23rd (June 10) to the 31st week (Aug. 5). It brooded about half as long as the previous two subsets. It terminated brooding at the same time as the very early group and 3 weeks earlier than the early group. Brooding began when rising spring water temperatures reached 21°C and ended at the onset of late summer cooling (24.5°C).

The final subset, late season brooder, included only one species: *Q. fragosa*. It had a very short 5.8-week brooding period extending from week 34.7 (Aug. 31) to 40.5 (Oct. 11). It brooded less than 75% of the duration of any other species. Brooding periods for all species from all four subsets overlapped except for *Q. fragosa.* This species had a distinct, late and short brooding period that did not overlap with any other amblemine (see Figure 2). Brooding began 4 weeks after the start of fall cooling when temperatures fell to 22.5°C and ended when temperatures cooled to 13.9°C. A summary of average projected brooding dates and temperatures is given in Table 2.

Table 2. Average Projected Brooding Dates and Temperatures.

Subset	Average Brooding Start		Average Brooding End		Average Glochidial Release Period
Very Early	April 21	10°C (rising)	July 21	25°C (peaked)	June 18 – July 29
Q. metanevra	April 18	9.5°C (rising)	Aug. 17	22.5°C (falling)	July 22– Aug. 17
Q. p. pustulosa	April 22	10°C (rising)	July 22	25°C (peaked)	June 3 – July 22
T. verrucosa	April 22	10°C (rising)	June 17	21°C (rising)	May 20 – June 17
C. tuberculata	April 22	10°C (rising)	July 29	25°C (peaked)	July 1 – July 29
Early	May 14	16°C (rising)	Aug. 16	22.5°C (falling)	July 14 – Aug. 26
E. dilatata	May 10	14°C (rising)	Aug. 5	24.5°C (peaked)	July 15 – Aug. 5
F. flava	May 20	17°C (rising)	Aug. 26	23°C (falling)	July 19 – Aug. 26
P. sintoxia	May 13	16°C (rising)	Aug. 16	23°C (falling)	July 8 – Aug. 16
Mid-Season					
A. p. plicata	June 10	21°C (rising)	July 29	25°C (peaked)	July 1 – July 29
Late-Season					
Q. fragosa	Aug. 31	22.5°C (falling)	Oct. 6	15.2°C (falling)	Oct. 1 – Oct. 6

An amblemine brooding in the fall is very unusual. We are aware of only one other amblemine that fall broods. *Megalonaias nervosa* (Rafinesque, 1820) broods during September through May (Gordon & Layzer, 1989). At northern latitudes, *M. nervosa* appears to brood from 25 August through 19 November (Heath, Engel and Holzer. 1988).

Gordon and Layzer (1989) provide a comprehensive summary of known mussel gravidity periods from a large number of investigations conducted at various North American locations. In general, our results agree with their summary with two exceptions. Ranges in brooding dates are larger in the 1989 summary (Figure 3). This is probably due to much samples from a wide range of geographic locations within which brooding dates may vary by latitude. Secondly, they reported gravid *Q. fragosa* during May; we found it gravid only during September and early October.

Gordon and Layzer's (1989) *Q. fragosa* brooding period summary came from Wilson and Clark (1914), the only original source reporting brooding for this species. We believe this record is from specimens of the morphologically similar *Q. quadrula*, not from *Q. fragosa.* Wilson and Clark (1914) reported gravid *Q. fragosa* during 17 and 29 May 1911 in the upper Cumberland River, upstream of Cumberland Falls. We now know that there are no reliable records of *Q. fragosa* from the Cumberland River upstream of Cumberland Falls (Stansbery, Pers. Comm.) and that there are reliable records of *Q. quadrula* from the upper Cumberland River.

Species Goodness of *Q. fragosa*

There has been some taxonomic disagreement regarding the species goodness of *Q. fragosa* (U. S. Fish and Wildlife Service. 1997). Some investigators have considered it simply an ecophenomorph of the more common *Q. quadrula*. The winged mapleleaf mussel recovery plan calls for taxonomic work to resolve this disagreement (U. S. Fish and Wildlife Service. 1997). In this investigation, we believe we have uncovered some information that suggests species goodness.

Winged mapleleaf brooding occurs late in the year (early Sept. through early Oct.) compared to nearly all other amblemines, including *Q. quadrula*. Gordon and Layzer (1989) report gravid *Q. quadrula* during April through August, which does not overlap or correspond to *Q. fragosa*. Therefore, assuming correspondingly non-overlapping spawning times, it appears that *Q. quadrula* and *Q. fragosa* are reproductively different and that genetic intergradation is very unlikely and are therefore reproductively isolated.

Q. fragosa growth

Data of pooled total length and mean length-at-annulus are give in Figure 4. Growth was nearly linear from age 1 to about age 16. After age 16 growth was asymptotic, approaching 90mm. The mean total length at the first annulus was 10.9mm and at the 22nd was 87.8mm.

Absolute growth from pooled length data and from recaptured mussels are given in Figure 5. Absolute growth decreased with age. Age 1 individuals grew about 8mm annually. By age 22 there was no measurable growth.

Absolute growth from pooled length data differed from absolute growth of those mussels that were recaptured. Nearly all recaptured mussels grew an average of about 2mm less annually than the general population. This represents a 60% decrease in annual growth. This suggests that handling or repeated handling of mussels may cause a decrease in growth rates. Speculatively, growth as measured by total length may have decreased for recaptured individuals because of retraction of the distal margins of the mantle. A retracted mantle margin would continue to produce new growth but not in the direction of total length. It is likely that this growth would be expressed in shell margin thickness, not total length, for a short time after retraction. Therefore, handling or repeated handling may not have affected growth as measured in biomass, which we did not do.

Effect of Climate on *Q. fragosa* Brooding Frequency

Frequency of brooding *Q. fragosa* individuals varied during the 1997-1999 10 Sept. through 8 Oct. brooding periods. During 1997, 3.7% of the sampling was found brooding (n = 28). During 1998, 21.3% was found brooding (n = 89). In 1999, 1.8% of the sample brooded (n = 56).

The substantially higher brooding frequency observed during 1998 may be due to warmer water temperatures relative to other years. From 9 Oct. 1997 to 9 Sept. 1998, the downstream mean daily water temperature was 11.8% greater than the mean from the same period during 1996-1999. Data summaries from other months show a similar pattern (Table 3).

Table 3. Mean water temperatures (°C) from the A. S. King Power Plant, Bayport, Mn.

Year	Time Period		
	9 Oct. – 9 Sept.	1 April – 9 Sept.	15 Aug. – 9 Oct
1996	11.83		
1997	9.46	17.26	19.32
1998	12.80	20.80	21.09
1999	11.72	19.47	18.54

Water temperatures may not be a controlling factor in brooding frequency but do seem to be correlated with it. This correlation could be a coincidence since only three years of data were collected. Also, other factors associated with water temperature, such as plankton productivity, discharge or heating degree-days could be more important. It would not be surprising, however, that water temperatures or

measures of heat would be important since this population of Q. fragosa is located at the northern edge of its historic and present geographic range. Additional analysis on this species' highly variable year class strengths and birth-year heat inputs may provide additional insight.

Size and Age at Sexual Maturity

During the brooding period, we examined individuals as small as 25 mm and as young as 3 years. The smallest of the 21 *Q. fragosa* individuals found gravid was 65 mm total length. The youngest gravid individual was 8 years. Because of our small sample size and the difficulty in determining gravidity, especially for smaller individuals, it is likely that sexual maturity occurs prior to age 8. Relative growth rates seem to decline from age 4 to age 6 (Figure 6). It has been suggested that these growth rate declines are associated with sexual maturity, at least in females. For other members of *Quadrula*, we have seen gravid individuals as young as age 3 but most seem to become sexually mature at age 5 or 6. Sexual maturity could be most accurately be determined by histological examination of the gonads which we did not do.

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Figure 1. General (Inset) and Specific Location of Study Area in St. Croix River, Polk County, Wisconsin and Chisago County, Minnesota. St. Croix Dalles, Wis.-Minn. (1978) and Osceola, Wis-Minn. (1993) USGS 7.5' quadrangles.

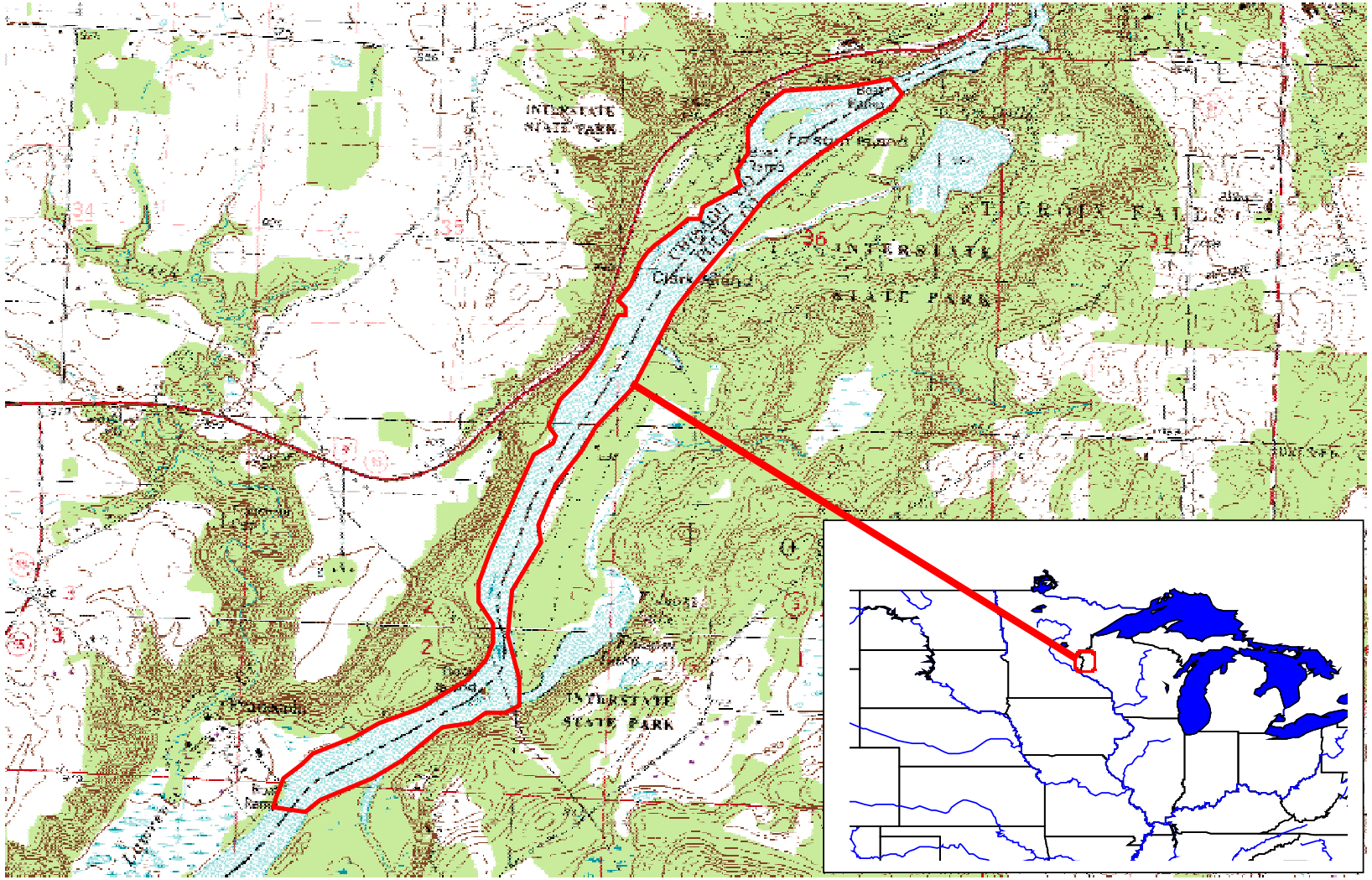


Figure 2. Brooding Period of Amblemines, Years 1997-1999 Combined.

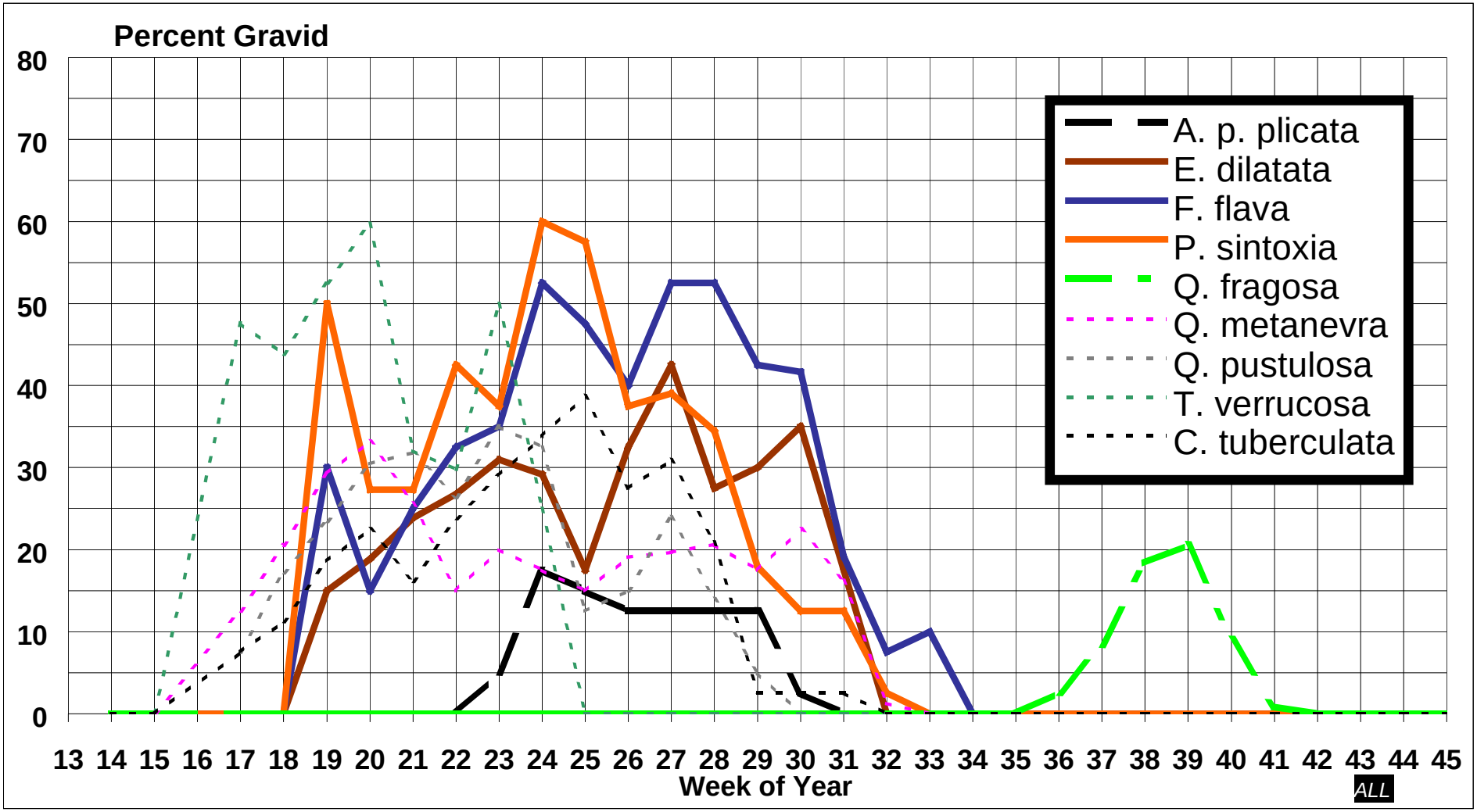
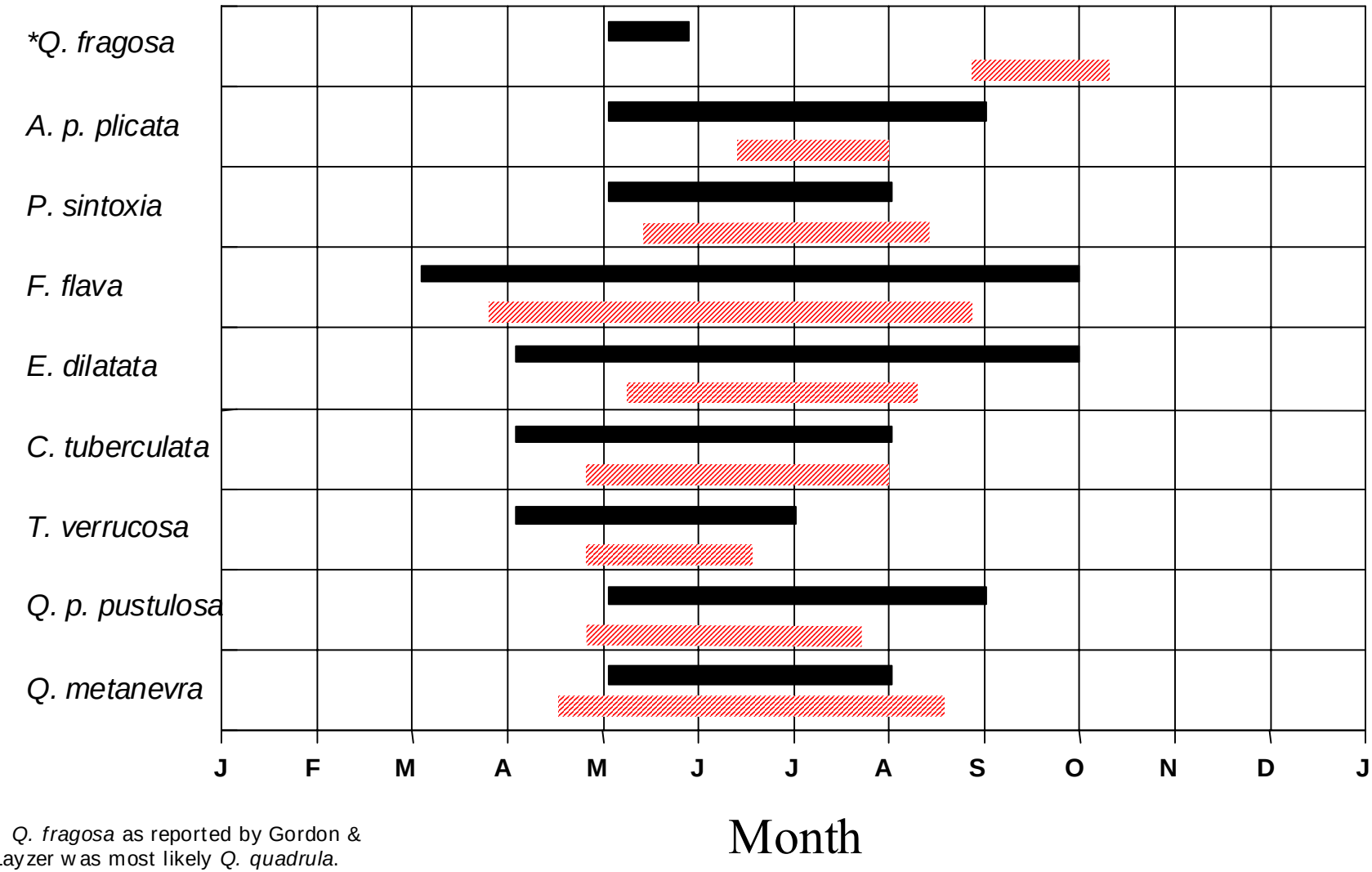


Figure 3. Comparison of Brooding Periods Between this Study (red patterned) and Gordon & Layzer (dark solid).



* *Q. fragosa* as reported by Gordon & Layzer was most likely *Q. quadrula*.

Figure 4. Q. fragosa, Mean Length at Age .

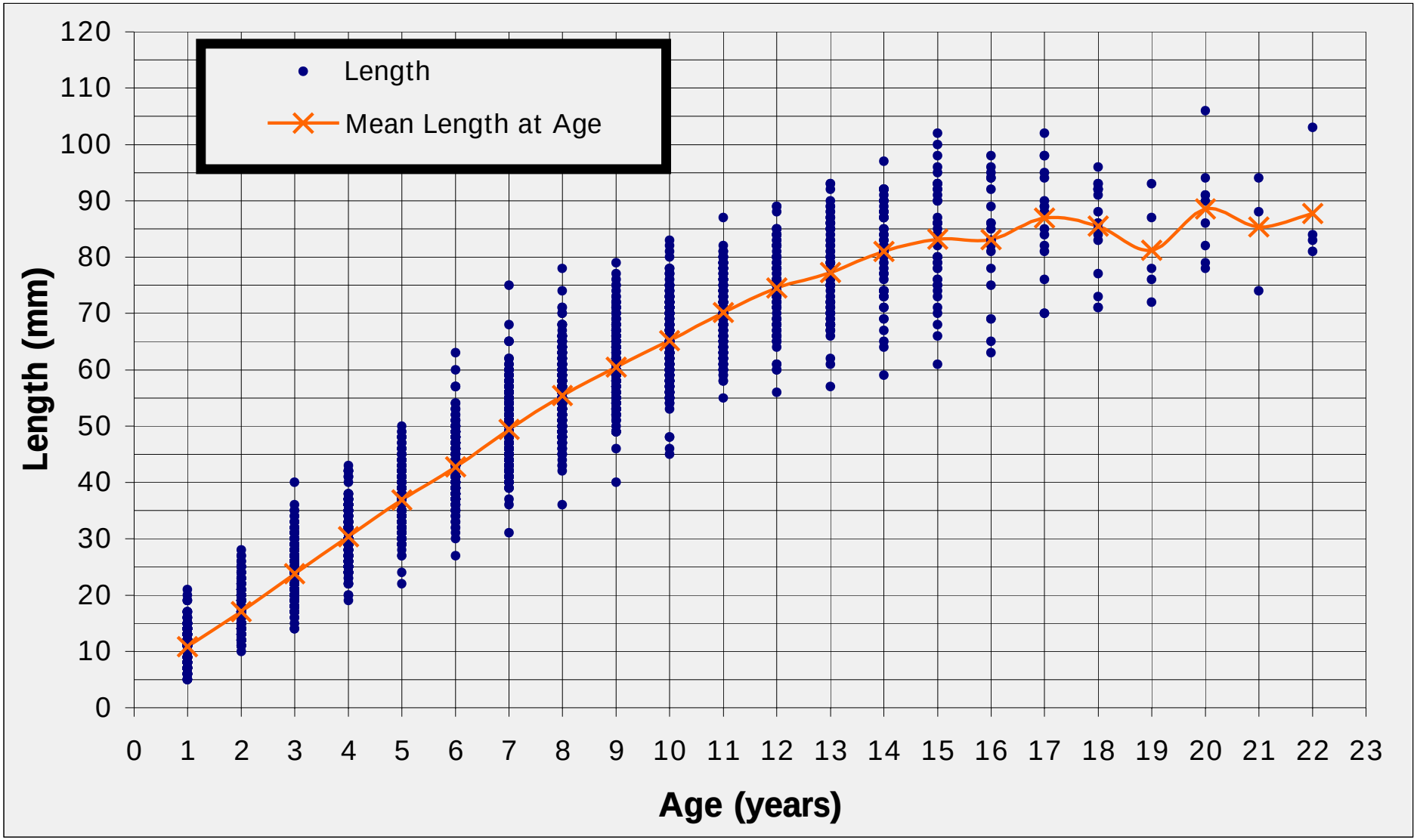


Figure 5. Absolute Growth of winged mapleleaf.

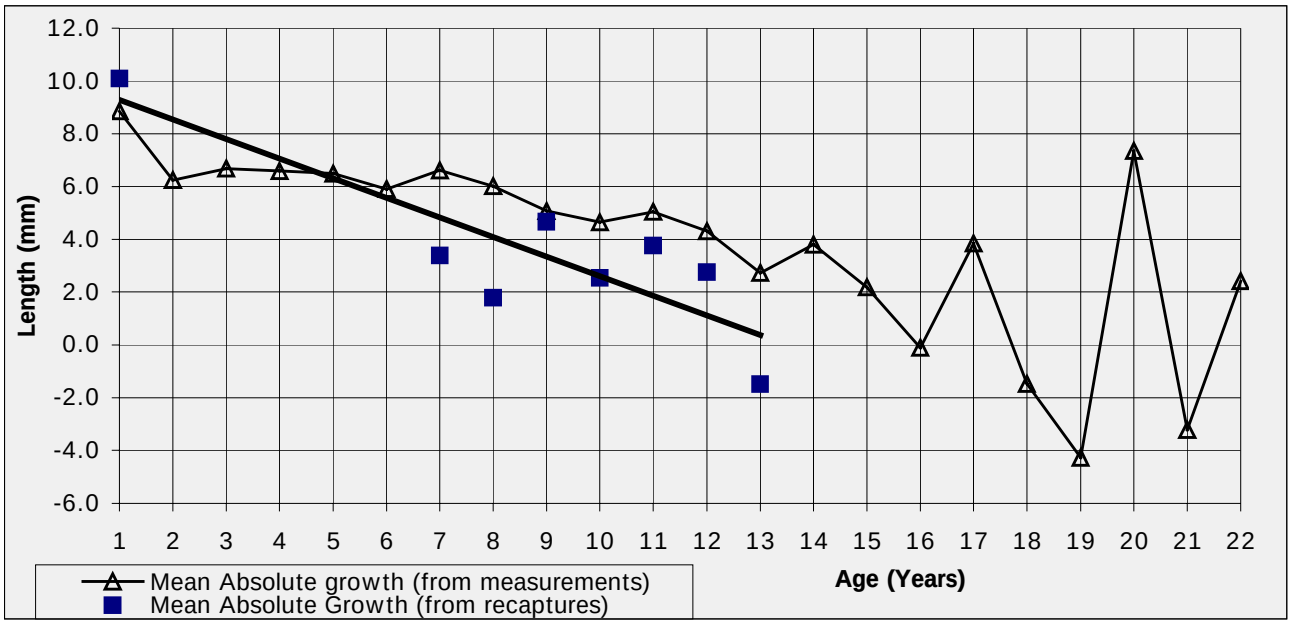
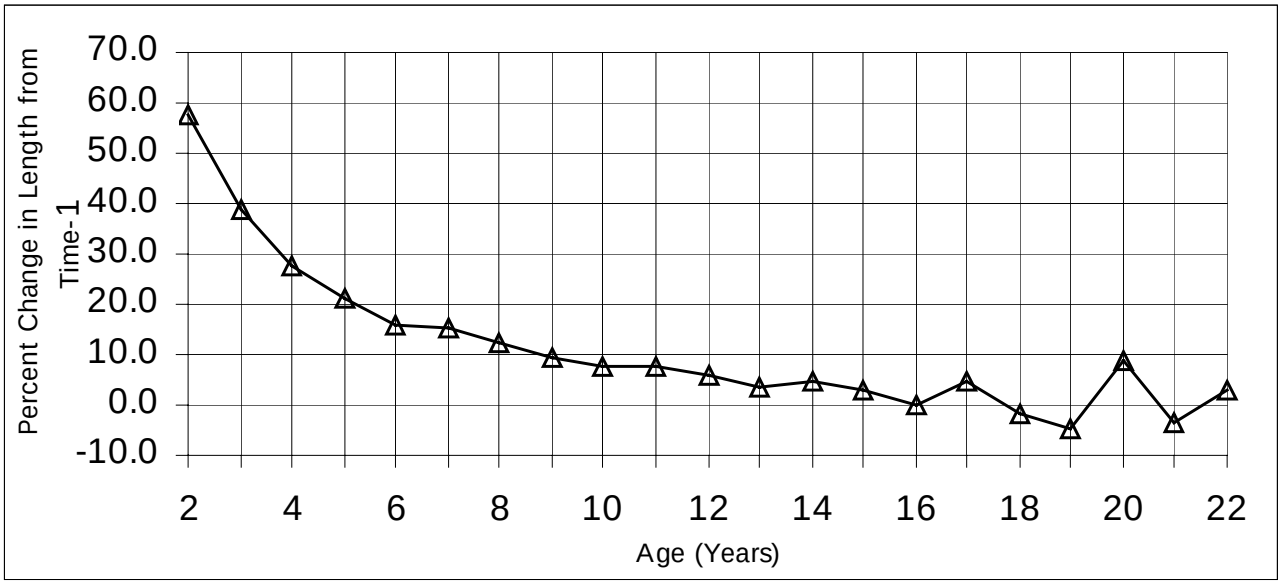


Figure 6. Relative Growth Rate of winged mapleleaf.



APPENDIX A.

Figures of Brooding Periods and Brooding Temperatures.

Figure A- 1. . Brooding Period of A. p. plicata, 1997-1999

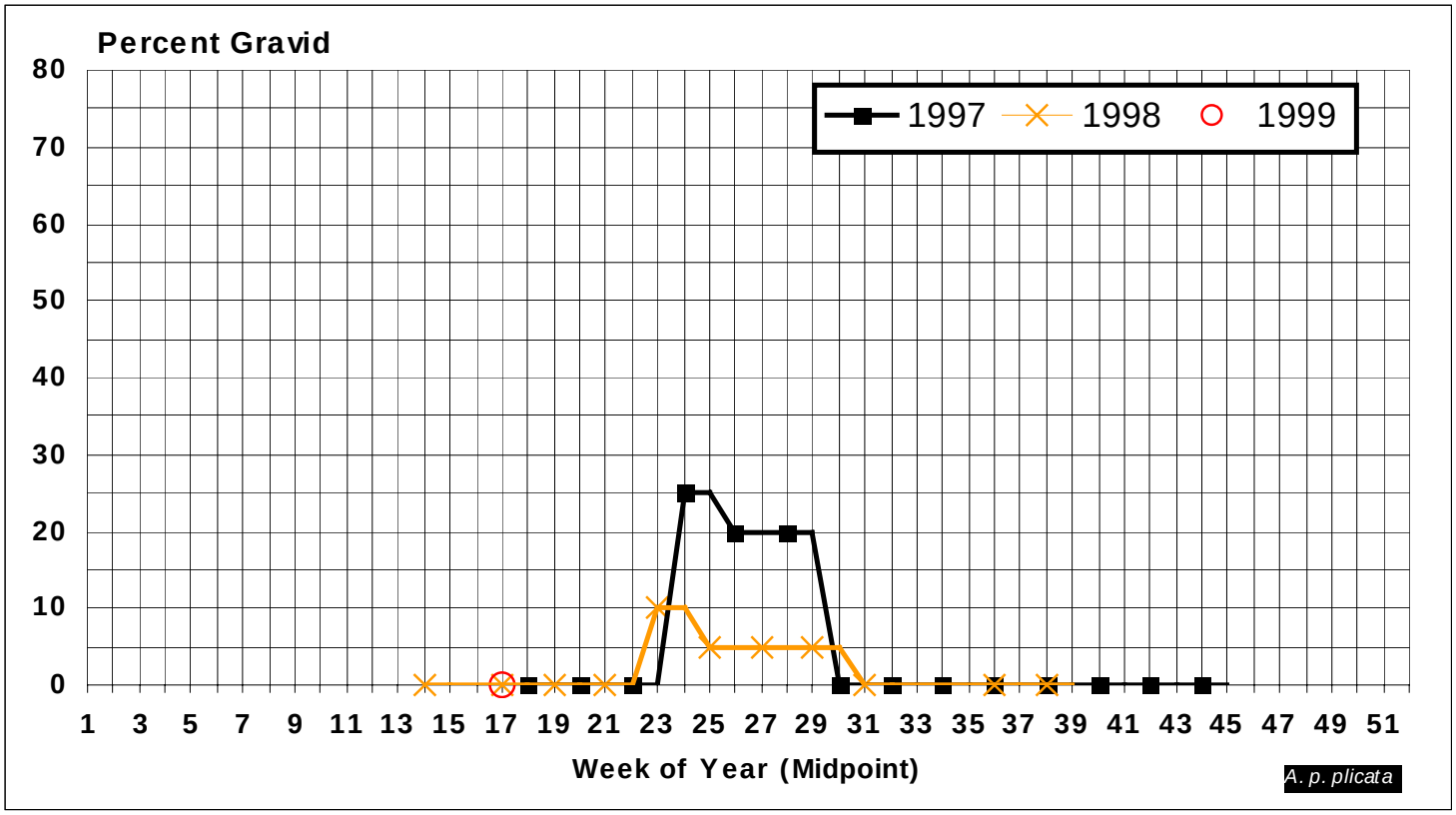


Figure A- 2. Brooding Period of C. tuberculata, 1997-1999.

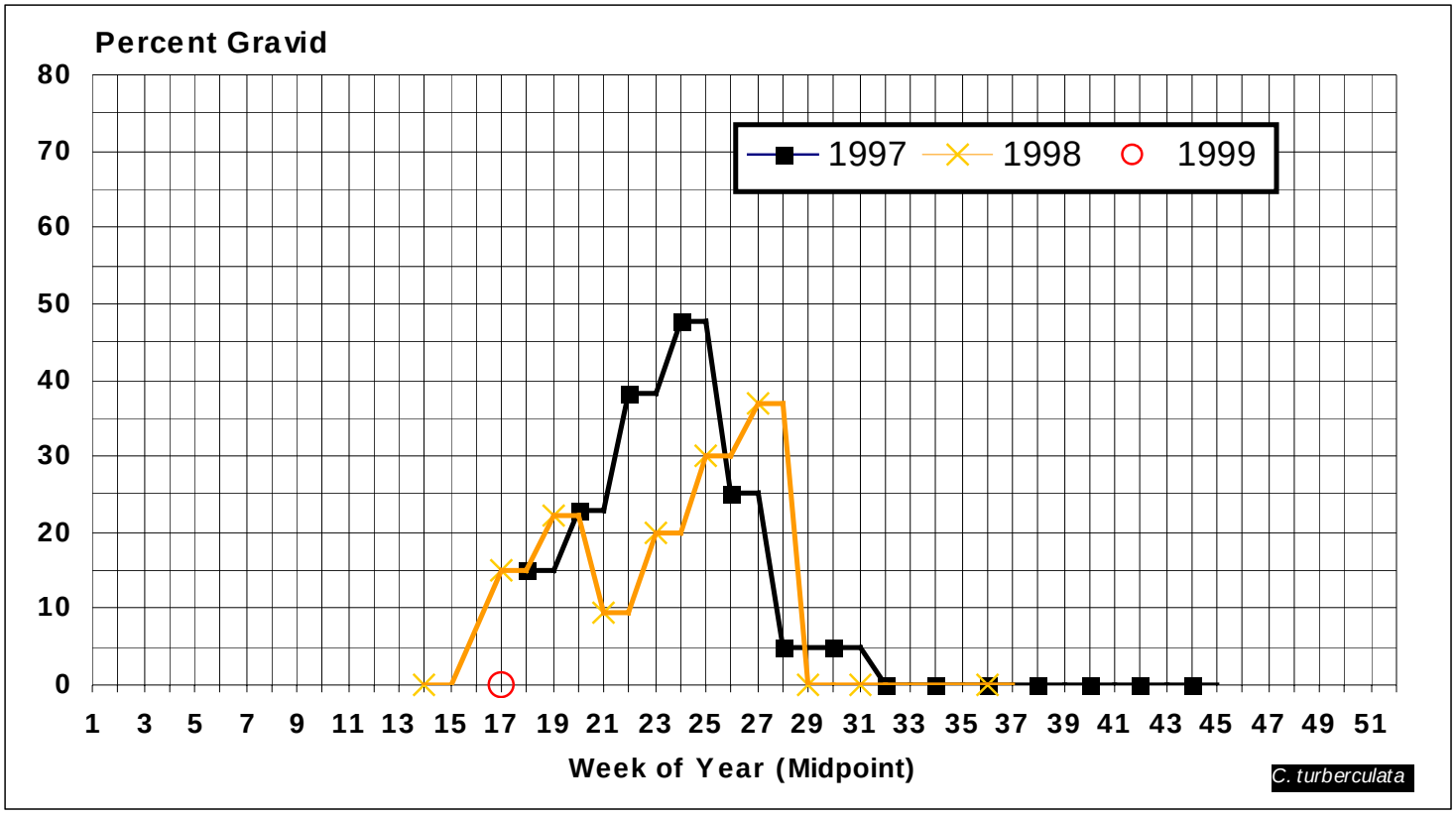


Figure A- 3. Brooding Period of E. dilatata, 1997-1999.

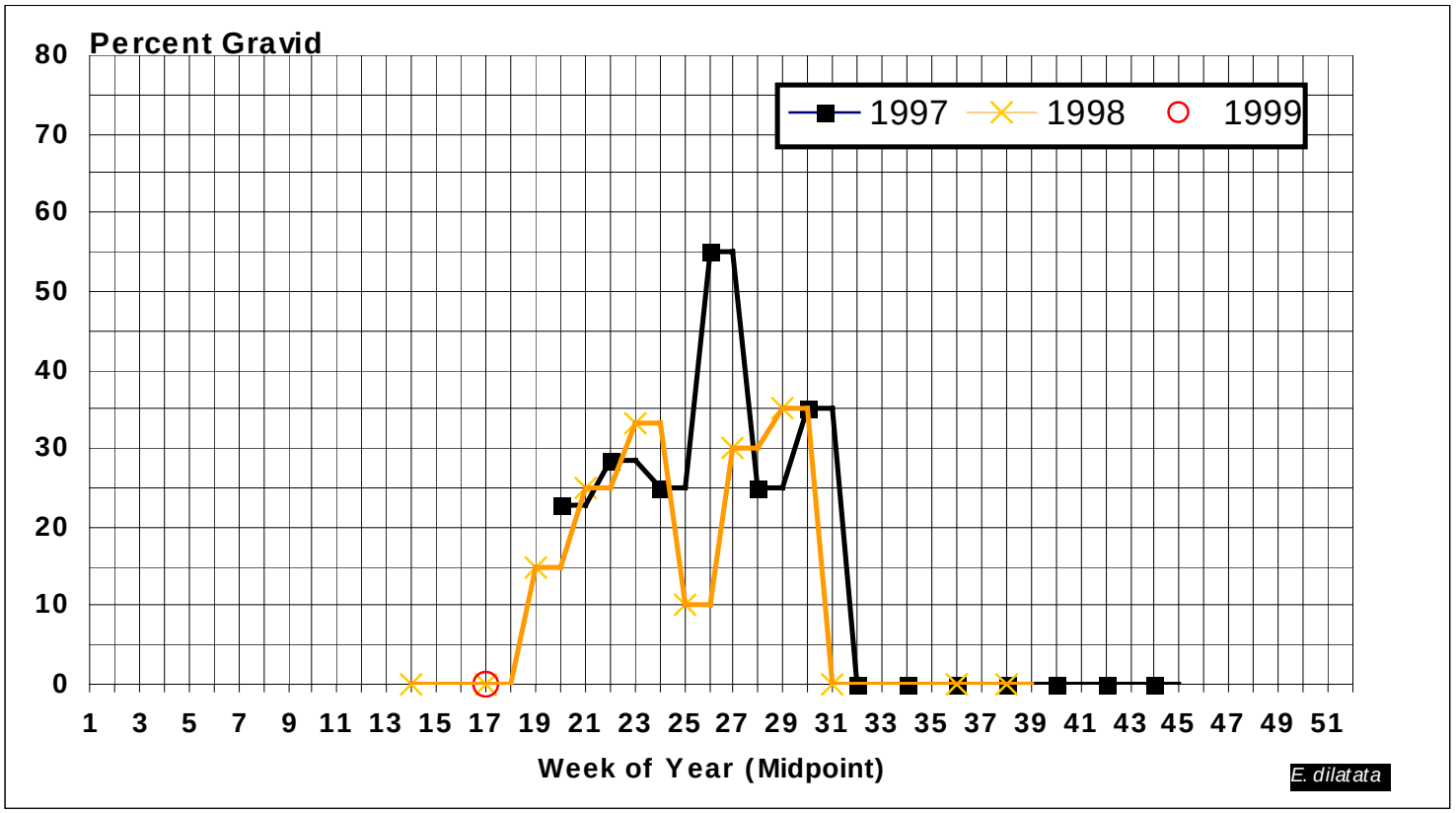


Figure A- 4. Brooding Period of F. flava, 1997-1999.

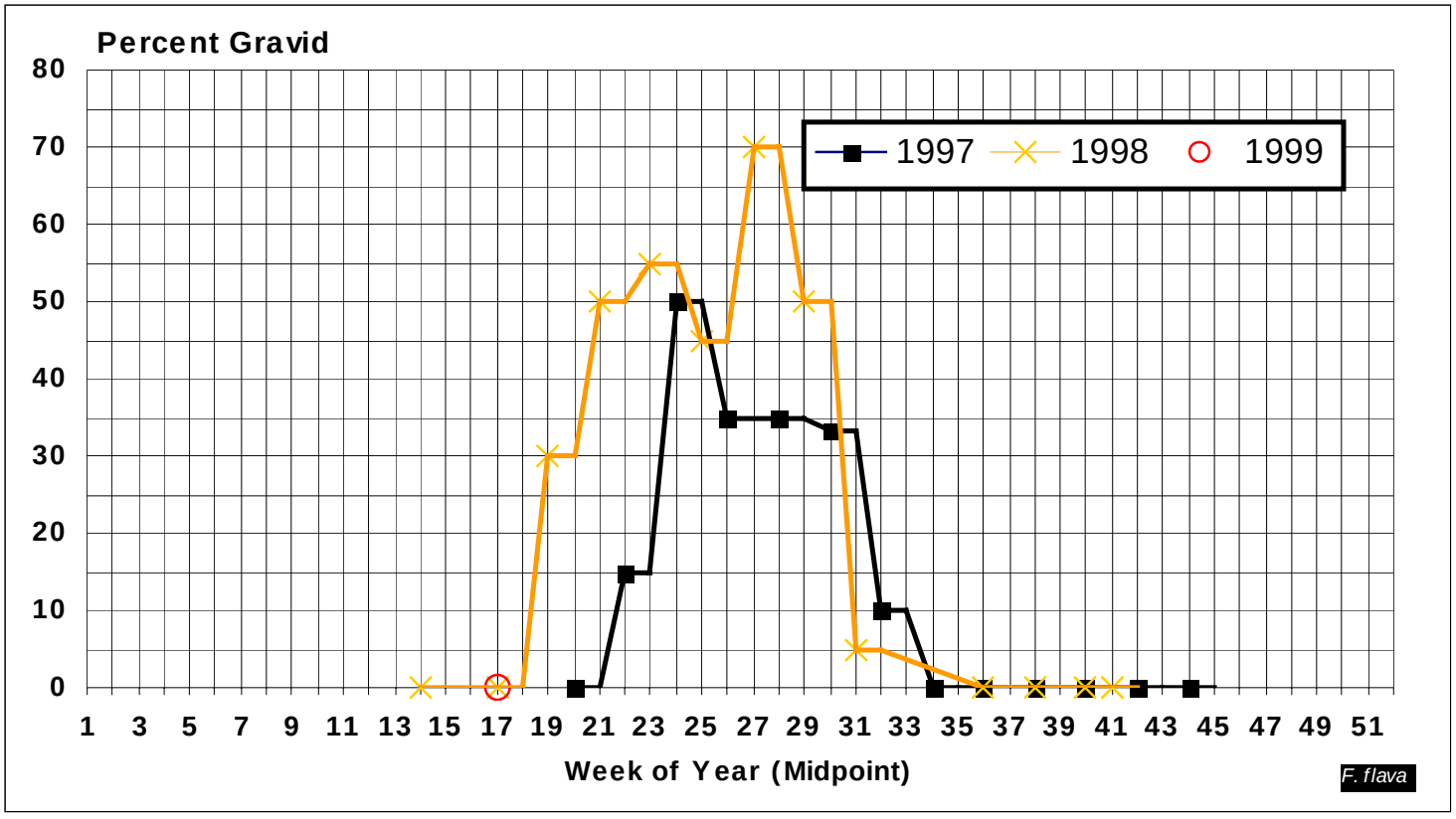
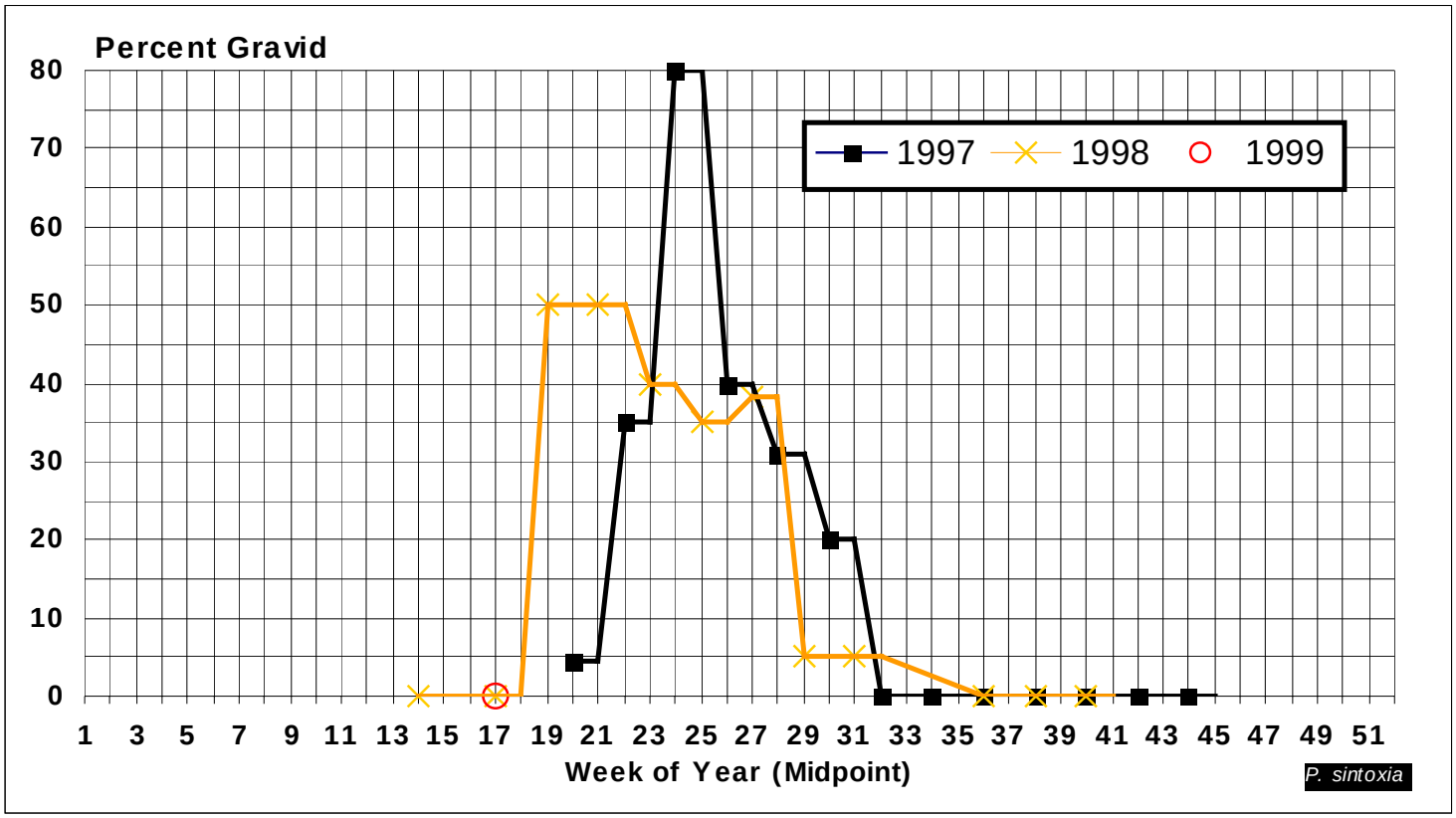


Figure A- 5. Brooding Period of P. sintoxia, 1997-1999 .



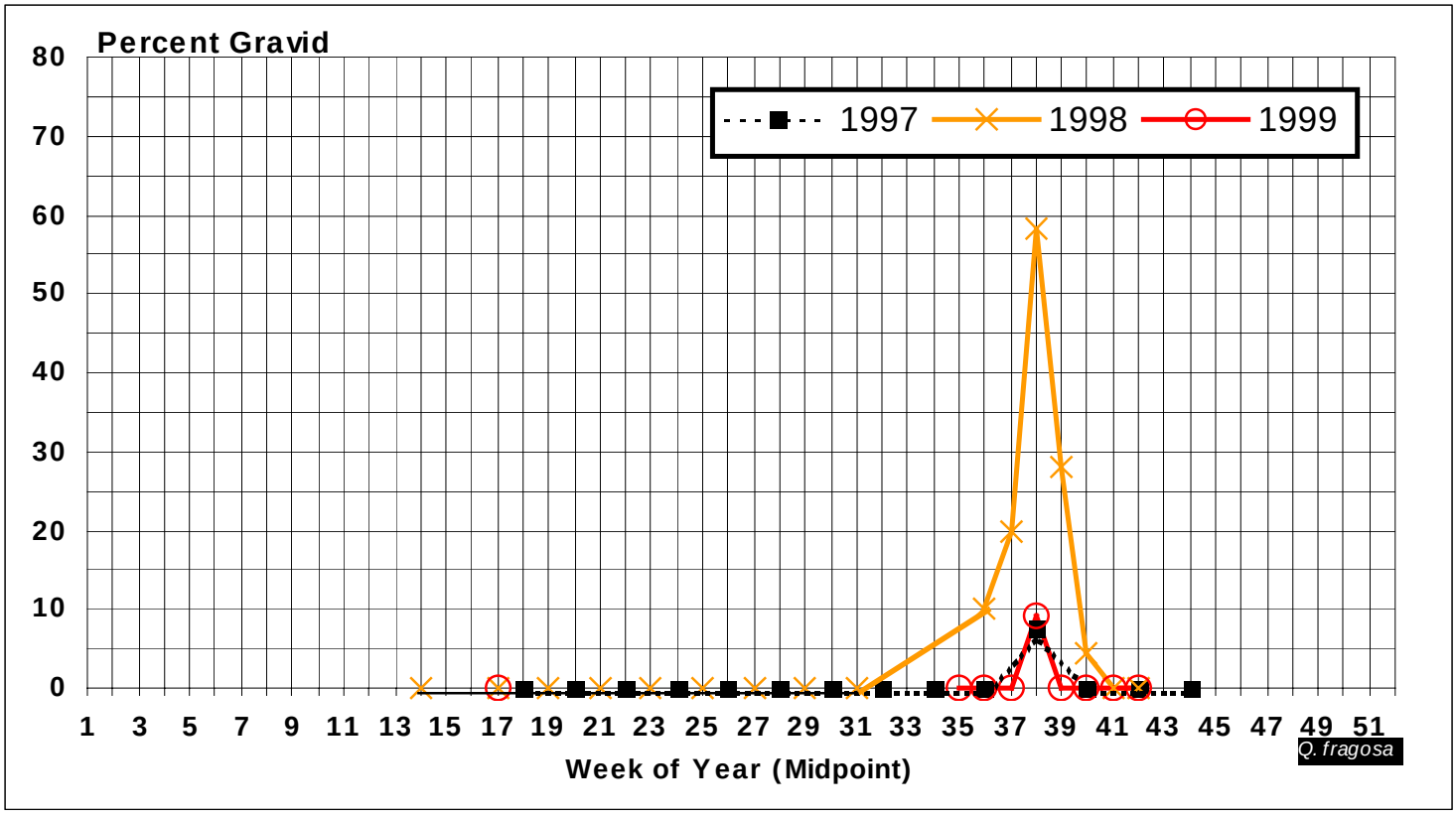


Figure A- 6. Brooding Period of Q. fragosa, 1997-1999.

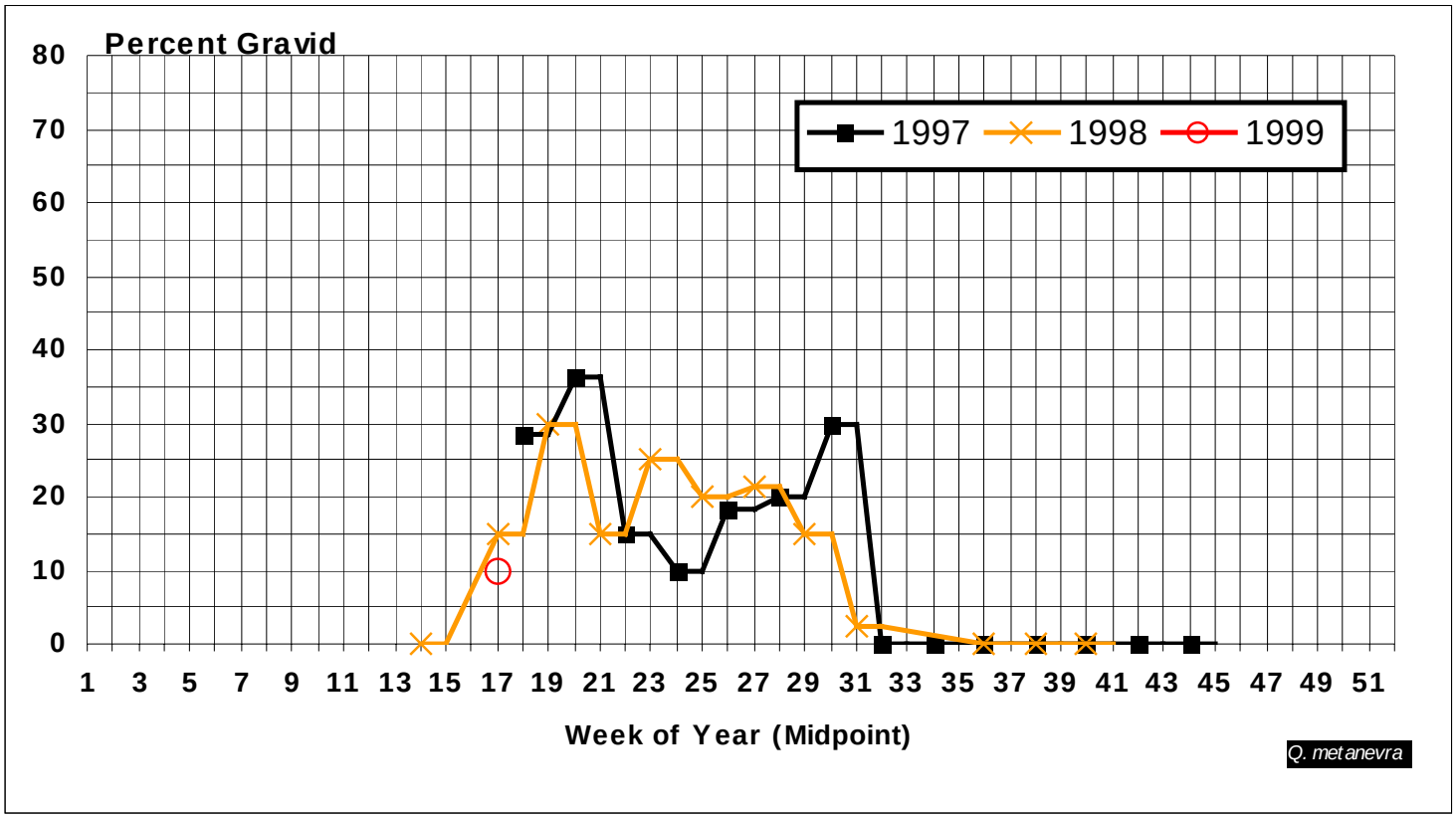


Figure A- 7. Brooding Period of Q. metanevra, 1997-1999.

Figure A- 8. Brooding Period of Q. p. pustulosa, 1997-1999.

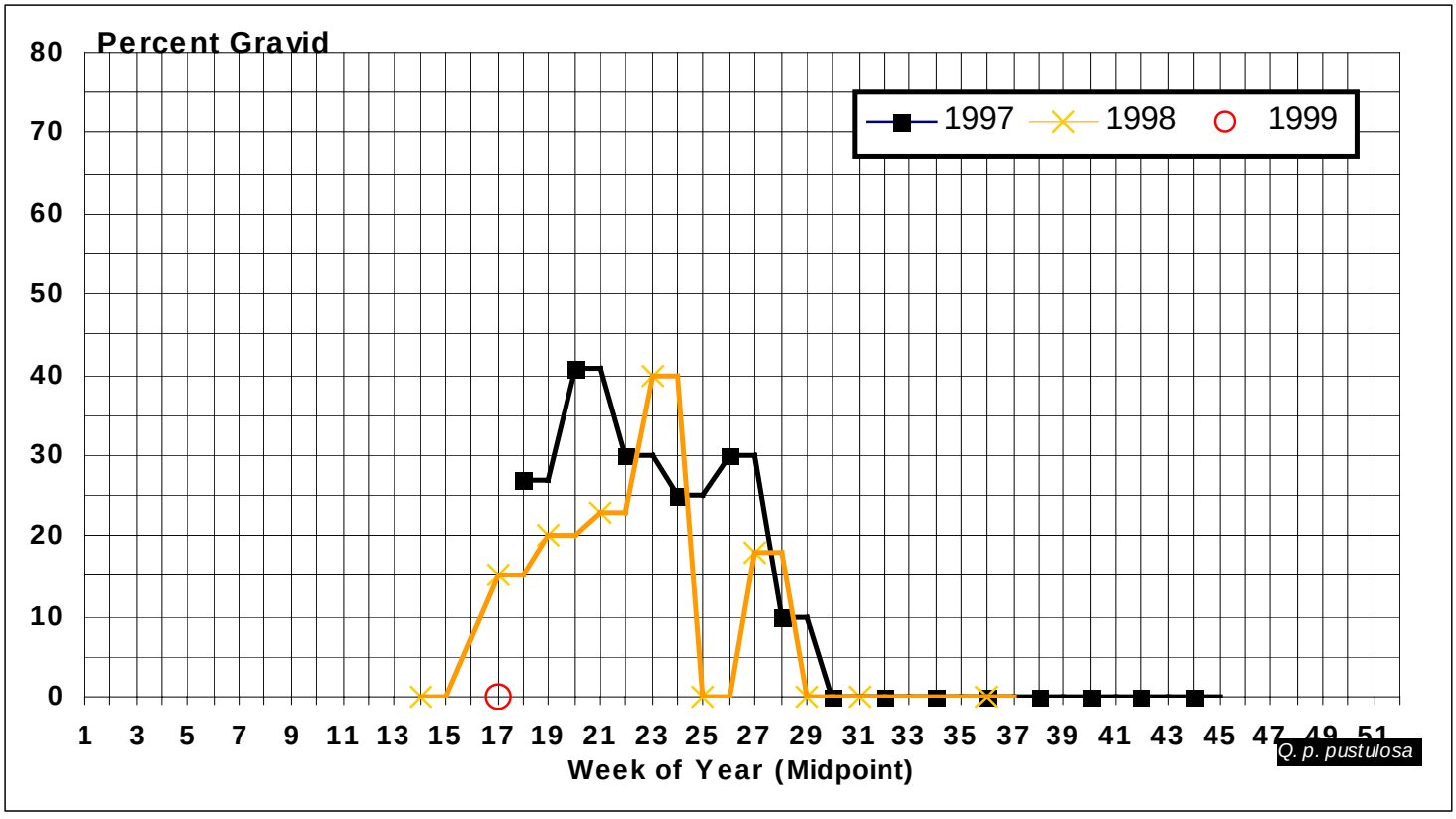


Figure A- 9. Brooding Period of T. verrucosa, 1997-1999.

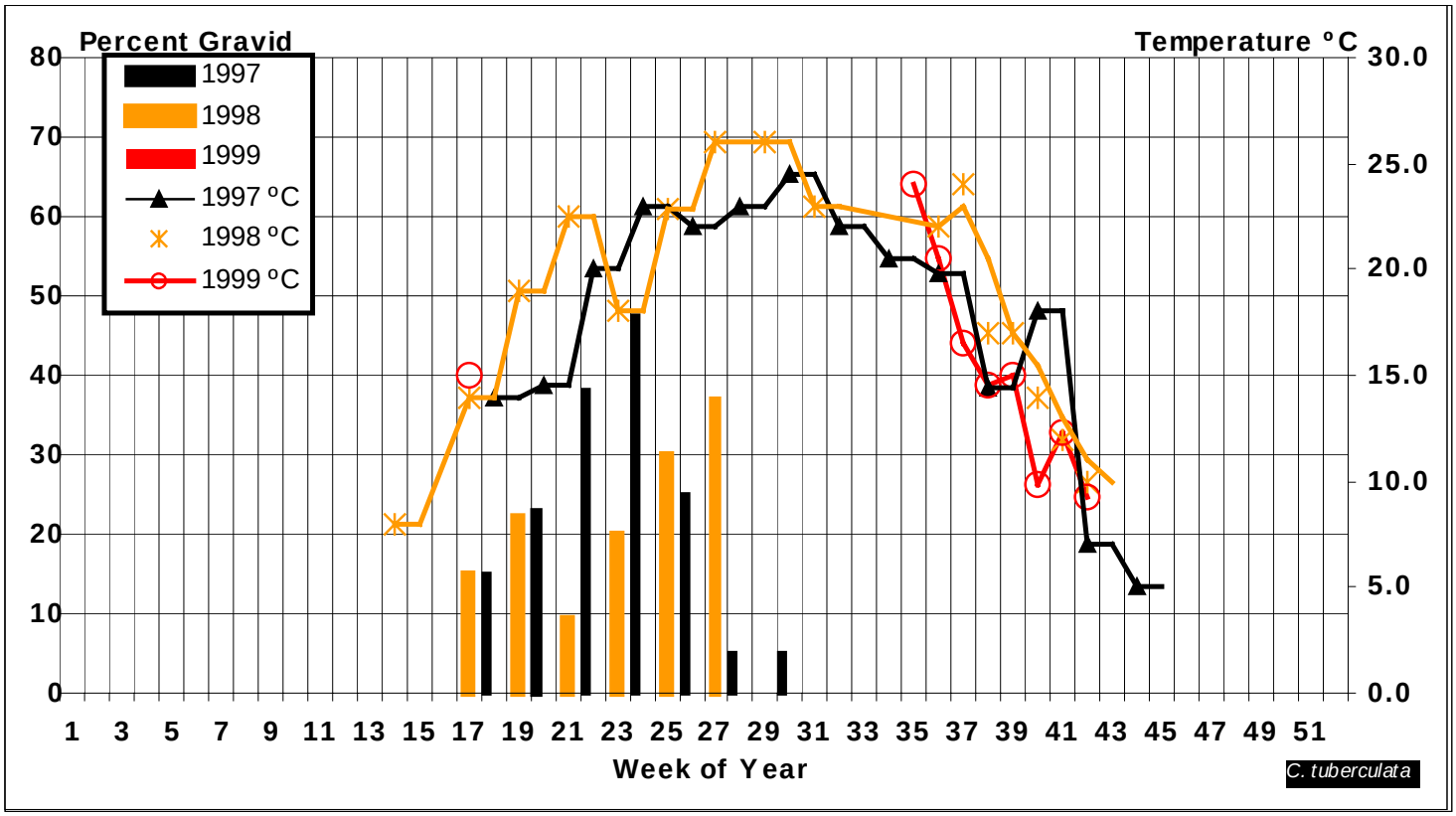


Figure A- 10. Brooding Temperatures of A. p. plicata, 1997-1999.

Figure A- 11. Brooding Temperature of C. tuberculata, 1997-1999.

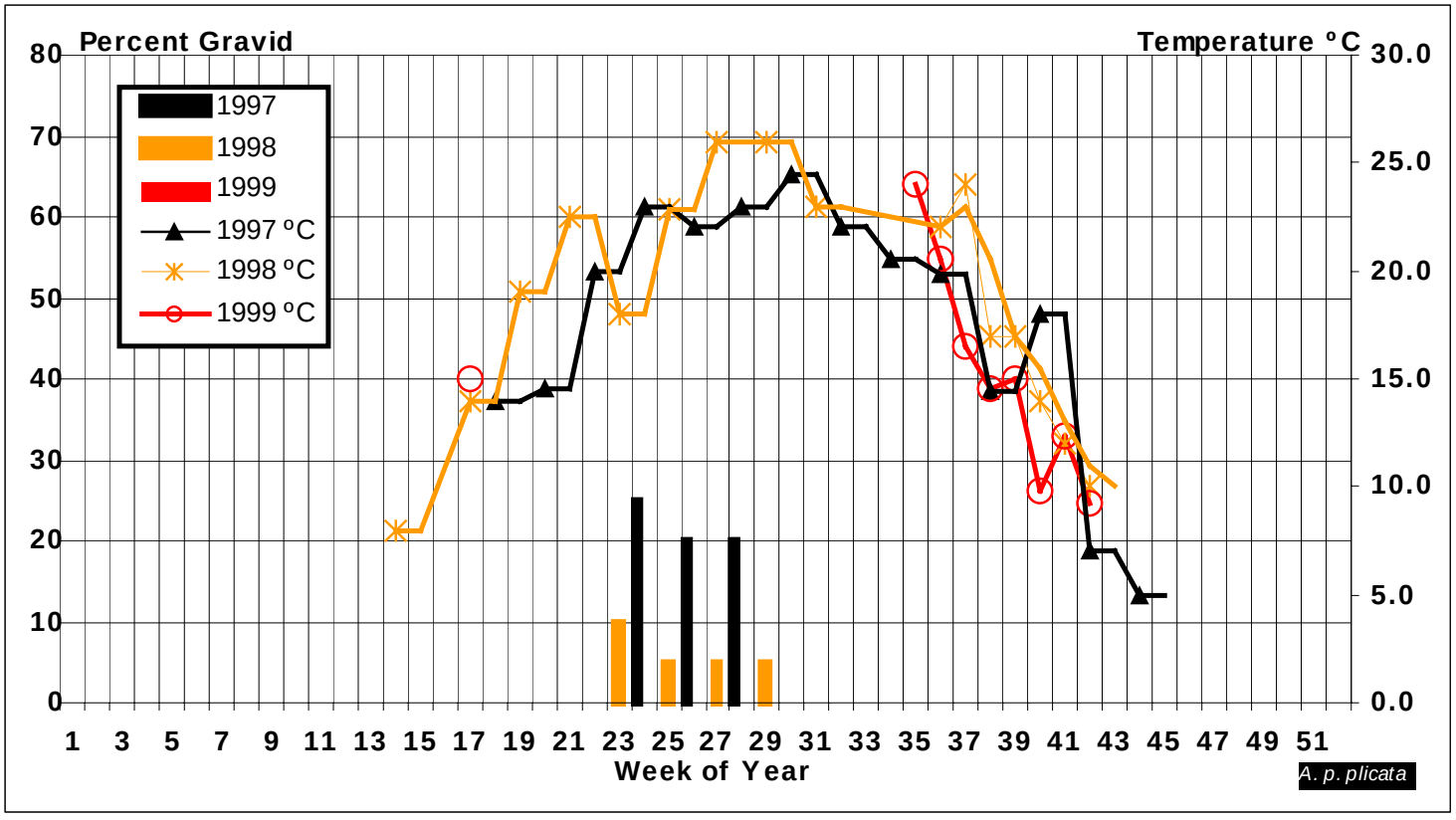


Figure A- 12. Brooding Temperature of E. dilatata, 1997-1999.

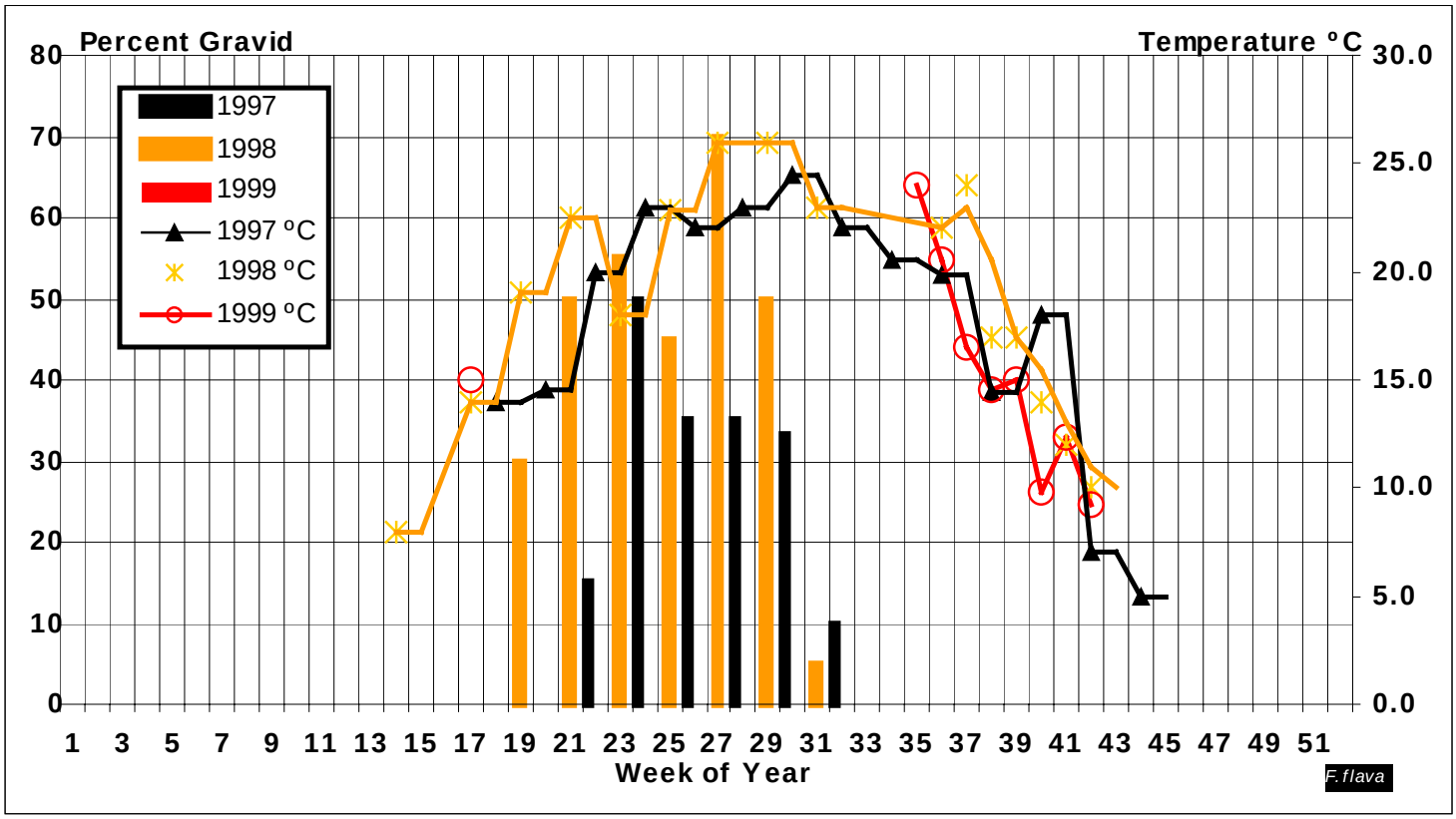


Figure A- 13. Brooding Temperature of F. flava, 1997-1999.

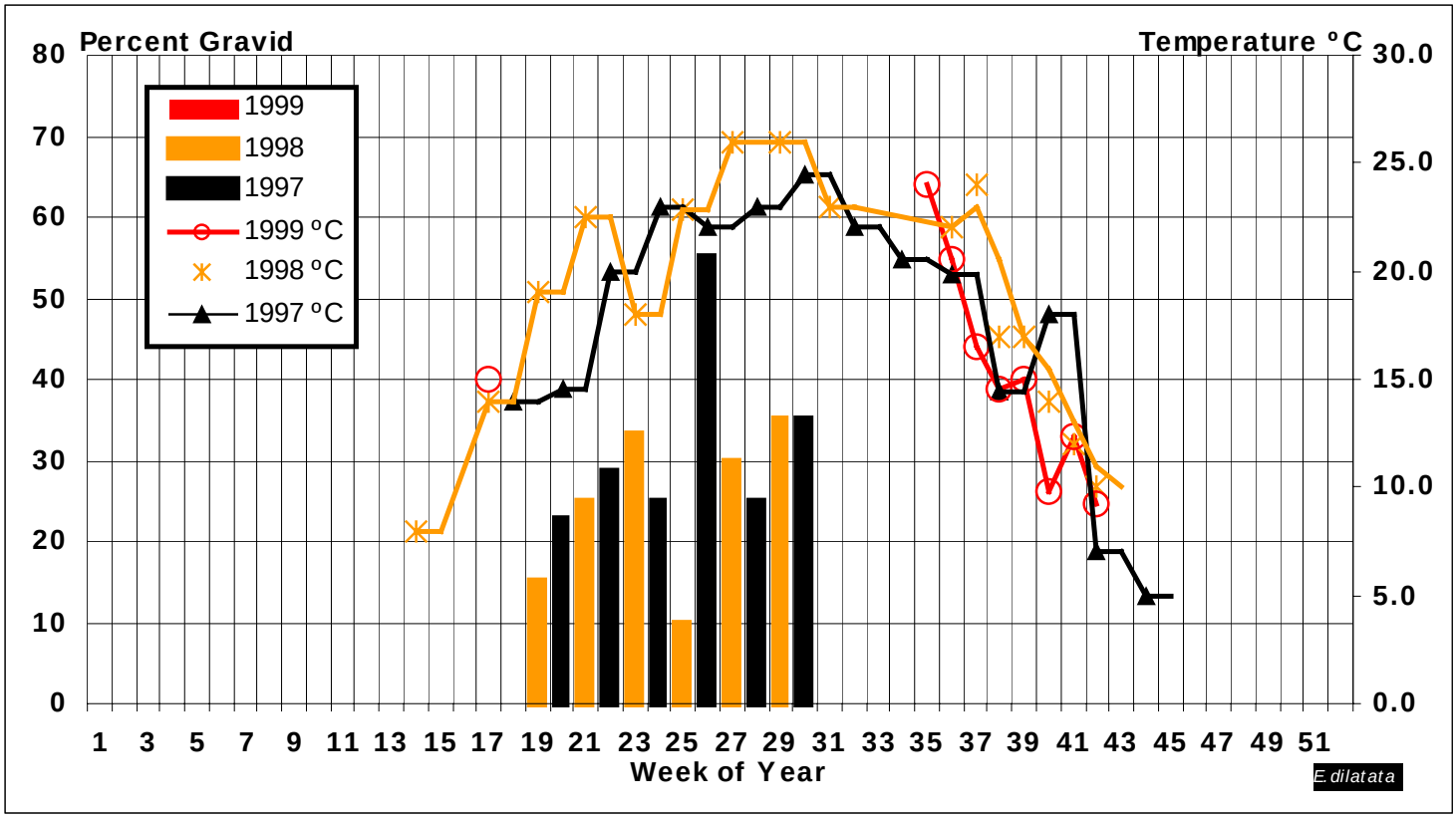


Figure A- 14. Brooding Temperature of P. sintoxia, 1997-1999.

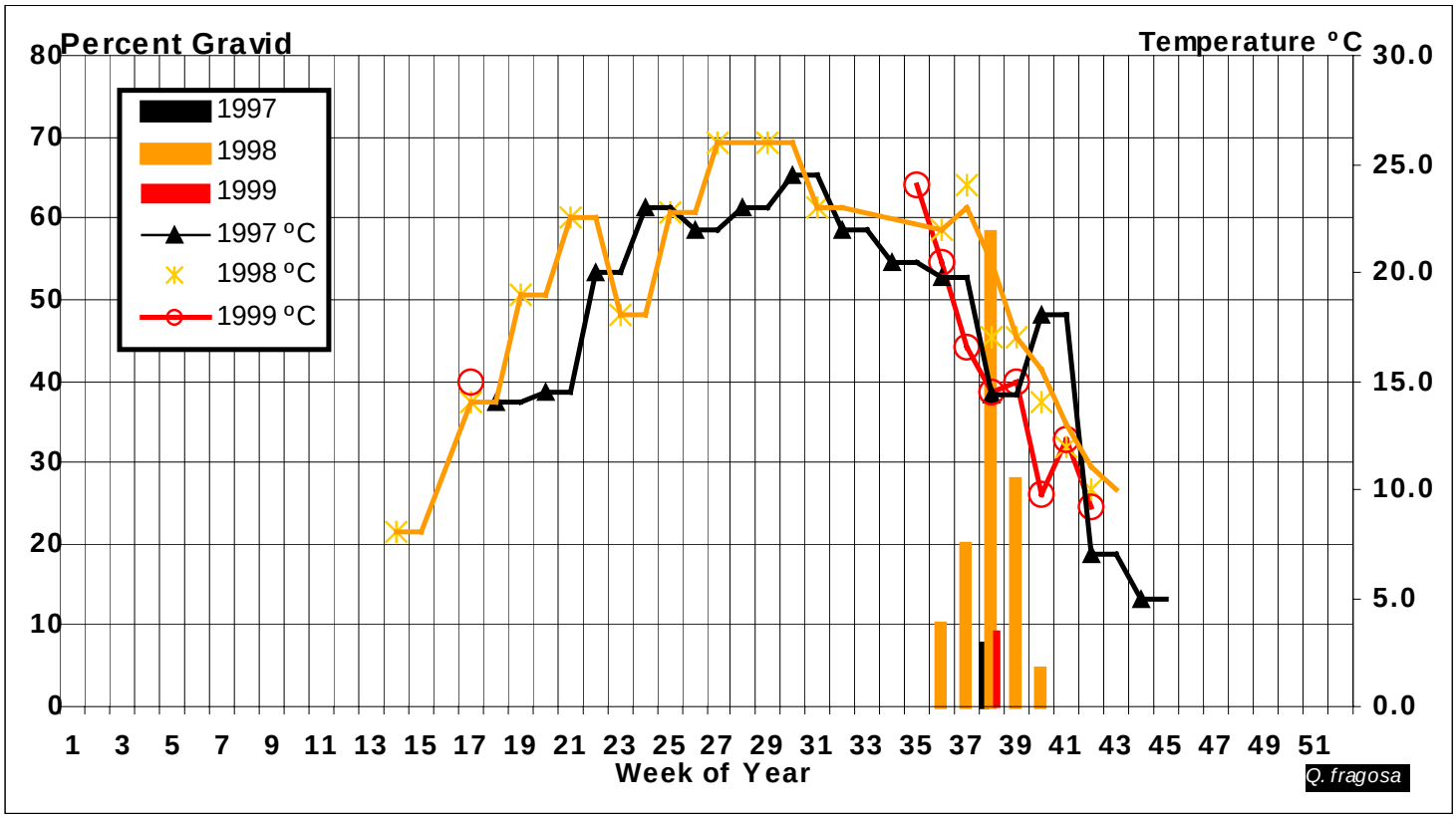
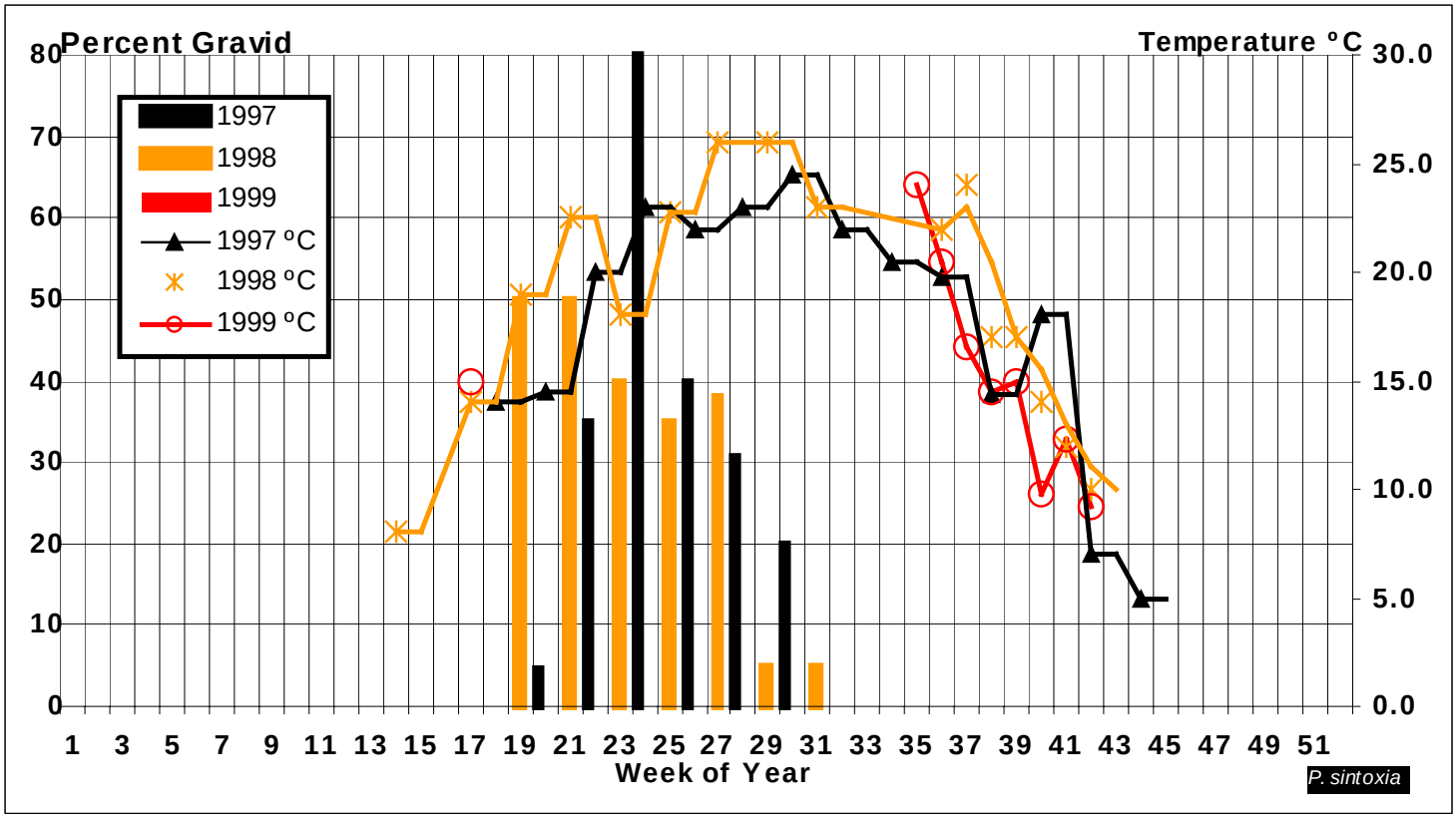


Figure A- 15. Brooding Temperature of Q. fragosa, 1997-1999.



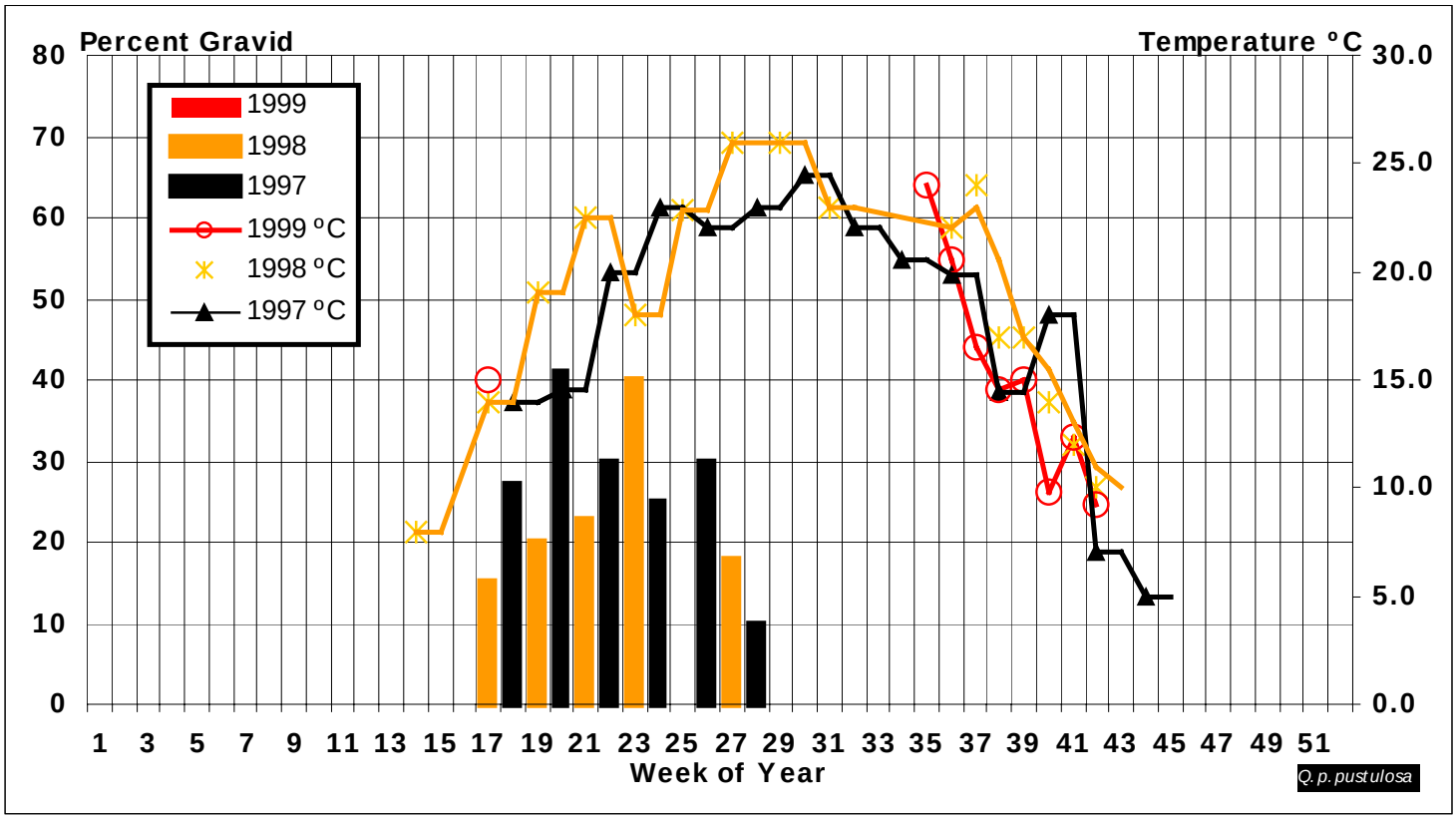


Figure A- 16. Brooding Temperature of Q. metanevra, 1997-1999.

Figure A- 17. Brooding Temperature of Q. p. pustulosa, 1997-1999.

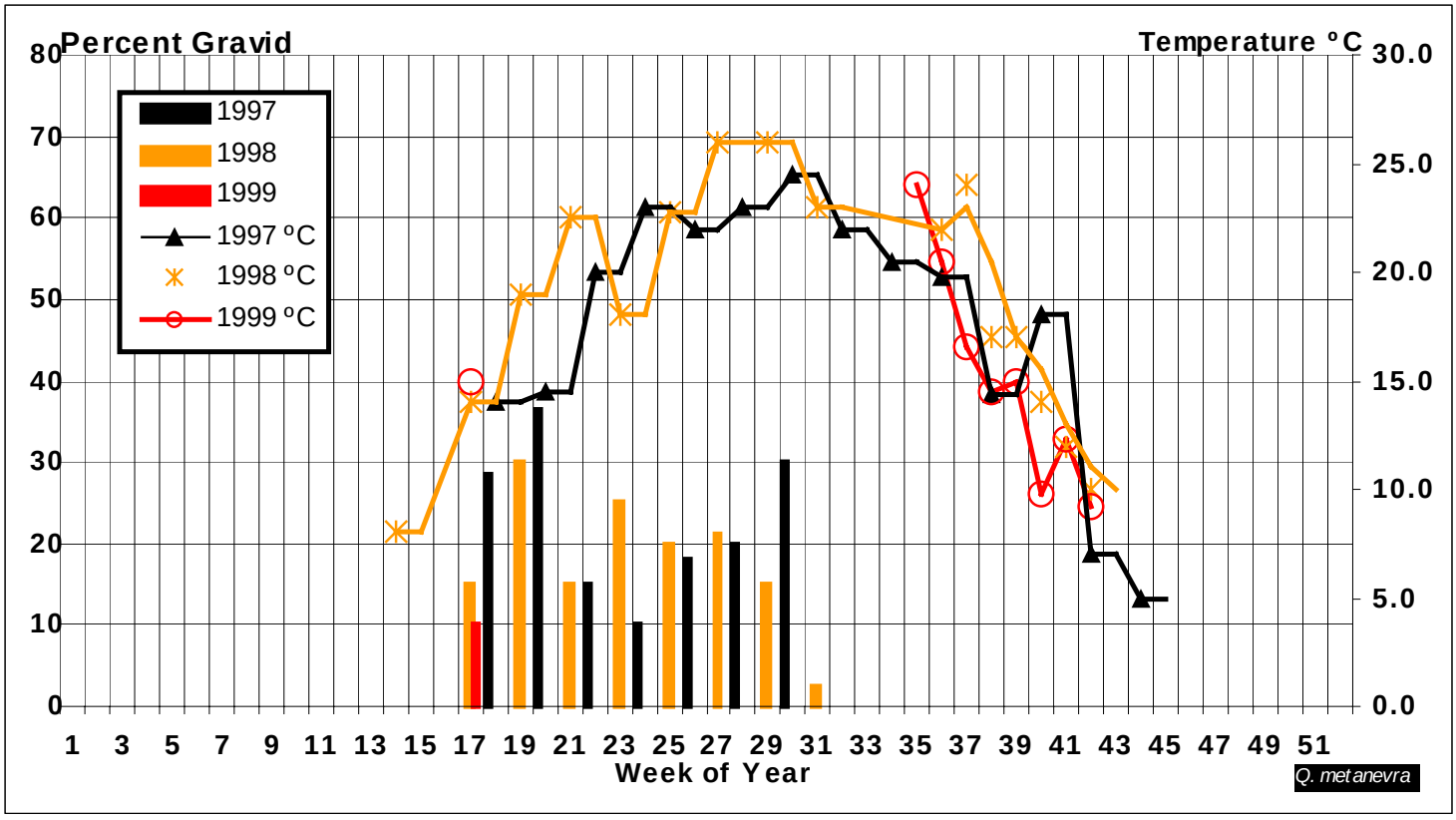
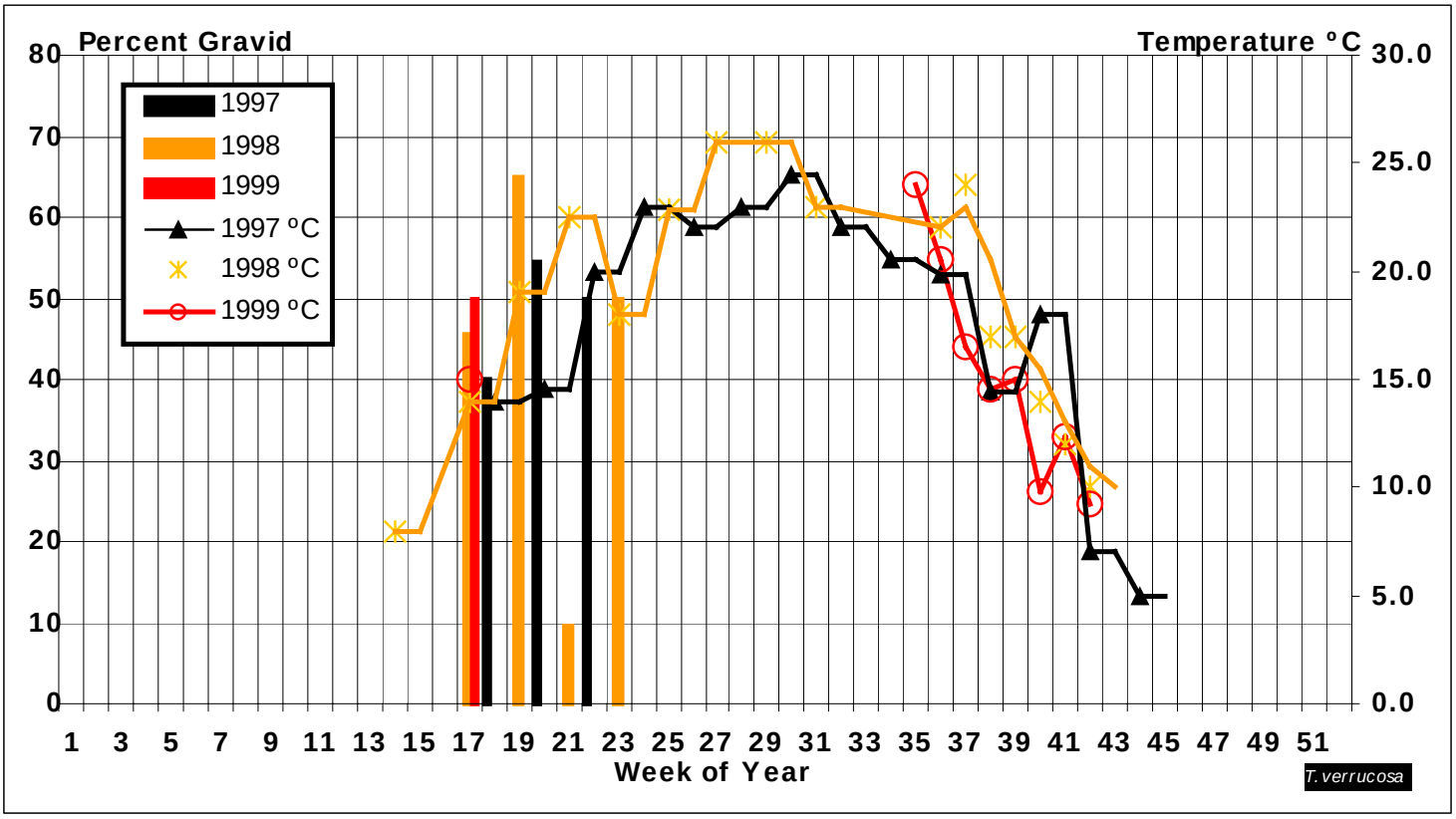


Figure A- 18. Brooding Temperature of T. verrucosa, 1997-1999.



Brooding Behavior and Suitable Host for the Winged Mapleleaf (*Quadrula fragosa*)

Final Report

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July 2000

Abstract

The winged mapleleaf (*Quadrula fragosa* (Conrad, 1835)) historically occurred in rivers across eleven U.S. states but now only occurs in a small portion of a few rivers. The glochidial host(s) for this federally endangered species are unknown which makes it nearly impossible to determine the viability of imperiled mussel populations either in degraded habitats, where they now occur, or in habitats being considered for relocation of mussels. We studied brooding winged mapleleaf in the St. Croix River and at the Wet Laboratory, University of Minnesota. During the brooding period a swollen excurrent aperture was observed among brooding and some non-brooding individuals. Glochidia were released individually or in conglutinates. Suitable glochidial hosts were determined using a standard artificial infestation protocol. Forty-five trials were conducted on thirty-five fish species or mudpuppies. Two juvenile winged mapleleaf were collected from a single channel catfish. Juvenile mussels grew substantially during the encystment period. We attempted to collect winged mapleleaf juveniles from naturally infested fishes but none of the recovered mussels were winged mapleleaf. Additional work is needed to determine the function of the swollen excurrent aperture displayed during the brooding season, and to verify that Ictalurids serve as glochidial hosts under laboratory and natural conditions.

Introduction

The winged mapleleaf (*Quadrula fragosa* (Conrad, 1835)) historically occurred in rivers across eleven U.S. states. Of two or possibly three populations thought to remain, the one in the St. Croix River bordering Minnesota and Wisconsin is at risk of zebra mussel colonization.

The host(s) for winged mapleleaf glochidia are unknown, although catfishes, perches, and sunfishes are hosts for its congeners (Watters 1994). Lack of information on mussel host requirements makes it nearly impossible to determine the viability of imperiled mussel populations either in degraded habitats, where they now occur, or in habitats being considered for mussel relocation. The original objective of this study was to identify suitable hosts for winged mapleleaf glochidia. During the course of the investigation we expanded the scope to include two additional objectives: (1) provide a brief description of adult brooding behavior, and (2) collect juvenile winged mapleleaf from naturally infested fishes.

Methods

Suitable fish hosts were evaluated using a standard protocol similar to that described in Neves *et al*. (1985). Most fish for artificial infestation trials were collected with a seine, angling gear, and electrofishing equipment from streams and rivers outside of the St. Croix River basin. This precaution was taken to avoid testing fish that may have been previously exposed to the species of glochidia under investigation and subsequently developed an immunity to subsequent exposures (Reuling 1919, Luo 1993). Test fish were held in flow-through holding tanks (40 L or 400 L). Holding aquaria water temperature was lowered at the same rate the St. Croix River cooled during the fall. Once water temperature reached 11 °C, the lowest water temperature available in the laboratory, infested fish were held at this temperature for the remainder of the winter.

Gravid winged mapleleaf mussels were collected from the St. Croix River in fall 1998 and 1999, held briefly in the laboratory, and returned to the river. All mussels were collected from and returned to the St. Croix River at Interstate State Park, Minnesota. Observations of brooding winged mapleleaf in the St. Croix River were collected by Ron Benjamin and Mark Endris, Wisconsin Department of Natural Resources. Gravid female mussels were brought into the laboratory and held in large beakers in flow-through aquaria, and released glochidia naturally (*i.e.* mussels were not excessively manipulated). Additional observations of brooding behavior were recorded in the laboratory and pictures and videotape was shot. To determine the health of collected glochidia, a small group of glochidia were exposed to a 0.1-1% sodium chloride solution. For each trial $\geq$ 70% of the glochidia closed their valves upon exposure to salt. Except for the glochidia collected from female #163, gravid females and glochidia that did not attach to test subjects were returned to the St. Croix River within 24 hr after collection. Glochidia collected from female #163 were not returned to the river because there was a small chance they were exposed to zebra mussel veligers. Glochidia from female #163 that failed to attach to potential hosts were frozen for future studies. Adult mussels that did not release mature glochidia were returned to the river one week after collection.

Fish were infested with glochidia by placing them in a 1-20 L bath with several hundred to several thousand glochidia under vigorous aeration. Fish were exposed to glochidia for 30 seconds to 3 hours, depending on susceptibility of the species to infestation. The state of infestation was assessed at 30, 60, and 180 seconds, and 5, 10, 30, 60, 120, and 180 minutes after initial exposure to glochidia. When a treated fish had at least 5-30 glochidia on its gills, it was transferred to a clean aquarium.

Infested fish were held in aquaria at the University of Minnesota Wet Laboratory. Most fish were fed frozen brine shrimp (*Artemia* sp.) three times a week. Fathead minnows (*Pimephales promelas*) were given to piscivorous fish once a week and removed from aquaria 5-10 minutes after introduction to minimize the possibility of their consuming glochidia or juvenile mussels lying on the aquarium floor. Small fishes (*e.g.*, cyprinids, etheostomids, young catostomids and ictalurids, *etc*.) were held in suspended nets to prevent them from eating juvenile mussels on the aquarium floor. Aquaria were siphoned and siphonate checked for presence of glochidia and juveniles every three days on average (range-two to ten days). Except for the lamprey, glochidia infestation levels were also checked every week. Due to the inaccessibility of the lamprey's gills, it wasn't possible to determine if the fish was infested, and if it was, to record the period of glochidial attachment. For this fish, the glochidial attachment period was determined solely through observation of glochidia in the siphonate. After 57 d the ammocoete was exposed to a lethal dose of anesthetic and dissected to determine if glochidia remained attached to its gill lamellae. Other infested fish were anesthetized and searched for attached glochidia using a dissecting microscope. If a glochidium was found, the fish was revived and the experiment continued until glochidia were no longer attached to the fish. Each search for juveniles was terminated when glochidia were no longer observed attached to the test subjects' gills and juveniles were no longer found in the siphonate. A mussel was considered a juvenile when foot movement or valve closure was observed. A fish was considered a suitable host when glochidia encystment and metamorphosis to the juvenile stage occurred. Glochidium descriptive nomenclature follows Hoggarth (1999). Fish, mussel, and amphibian nomenclature follows Robins *et al*. (1991), Turgeon *et al*. (1988), and Oldfield and Moriarty (1994) respectively.

To identify the fish species parasitized by winged mapleleaf under natural conditions we sought fishes overwintering in deep pools at Interstate State Park. Divers from the St. Croix National Scenic Riverway, National Park Service provided logistic and SCUBA diving support for fish capture with a dip net in a deep pool (approx. 50 ft) in the dalles area near Taylors Falls, MN. Fish were checked briefly for glochidial infestation in the field and likely candidates were brought to the Wet Laboratory. These fishes were held at 11 °C and aquaria were siphoned weekly to determine if juvenile mussels excysted.

Results

Winged mapleleaf exhibited several interesting behaviors while brooding glochidia. Unlike most Amblemines, which brood during spring and summer, winged mapleleaf embryos develop during a relatively short period in September and October. During this period SCUBA divers noticed that a greater proportion of winged mapleleaf were positioned higher in the substrate (*i.e*. more exposed) than during other times of the year. Additionally, we were surprised to see brooding and some non-brooding individuals with a swollen excurrent aperture. The aperture protruded approximately 10 mm from the shell margin and had black-ridged crenulations overlaying the gray mantle. (Figure 1). On a few occasions we visited brooding mussels in the laboratory late at night to see if there were changes in brooding behavior. No obvious differences were observed. Demibranchs of gravid winged mapleleaf were usually only slightly swollen making non-lethal gravidity determinations difficult. Consistent with other amblemines, all four demibranchs served as marsupia. On two occasions we observed two different brooding mussels release conglutinates individually and in groups from their swollen excurrent aperture (Figure 2). Glochidia were densely packed throughout the length of the elongate conglutinates. Conglutinates were approximately 1 cm long and 2 mm wide, white, and tapered at both ends (Figure 3).

Many characteristics of winged mapleleaf glochidia were visible in images captured using a scanning electron microscope. Glochidial valves are subelliptical (Figure 4), and overlap when fully adducted. There is little if any gap between the valves (Figure 5). From the micrographs we took it is difficult to determine if there are short dorsal alae or not. No hook is present and lanceolate micropoints appear to be unorganized along the ventral margin (Figure 6). Exterior surface sculpture was not photographed carefully enough to describe it properly.

Several brooding winged mapleleaf released glochidia in the laboratory. In 1997 a brooding winged mapleleaf was brought into the laboratory where it released incompletely developed glochidia. The immature glochidia were unsuitable for host suitability trials and were frozen for future use since the glochidia will not mature outside of the female. A number of gravid winged mapleleaf released their glochidia in the laboratory in 1998. On September 10 female #236 released a large number of glochidia in the field and laboratory. These glochidia were used to infest several fish species and mudpuppies. On September 18 two gravid females (#242 and #188) were brought into the laboratory. Female #242 released immature glochidia that were not usable for the study. Since the immature glochidia would not develop or survive if returned to the St. Croix River, they were frozen for future studies. Female #188 did not release glochidia. On September 24 females #149 and #102 were brought into the laboratory and 10 conglutinates from both females #117 and #154. Finally, female #163 was brought into the laboratory on October 8 with a small number of conglutinates she had released in the field. During the fall of 1999 only one gravid winged mapleleaf was observed. Female #193 released glochidia on September 28. These glochidia were used to infest all the fishes tested in 1999.

Winged mapleleaf glochidia host suitability tests were conducted on a variety of candidate species. Forty-four trials conducted on thirty-five fish species or mudpuppies were negative (Table 1). Thirty-eight trials on thirty species were conducted in 1998 and six trials using six species in 1999. In 1999 two juvenile winged mapleleaf were collected from an artificially infested channel catfish from the St. Croix River (Table 2). Unfortunately the catfish died just as juvenile mussels were beginning to excyst. Several pre-metamorphosed juveniles (unmoving, juvenile-like mussels) were collected from this fish just before its death. The pre-metamophosed juveniles had tripled to quadrupled in size. Although no juveniles were collected from other channel catfish, flathead and blue catfish, and yellow, brown, and black bullhead, sloughed glochidia had doubled or tripled in size during attachment (Figure 7).

In 1999 juvenile mussels were recovered from St. Croix River fishes naturally infested with glochidia. We collected an infested channel catfish and lake sturgeon from Interstate State Park (Table 3). Sloughed glochidia were recovered from the channel catfish and over 100 juveniles from the sturgeon. None of the juveniles collected had glochidial valves small enough to be considered winged mapleleaf.

Discussion

The inflated excurrent aperture observed among some winged mapleleaf during the brooding season shares characters with apertures observed among some other Amblemine mussels. Certain individuals of pistolgrip (*Tritogonia verrucosa*), purple wartyback (*Cyclonaias tuberculata*), and monkeyface (*Quadrula metanevra*) have inflated excurrent apertures during the brooding season (unpublished laboratory observations, Mark Hove, University of Minnesota). The purpose of these inflated structures is unclear. We have initiated studies at our laboratory to determine if the structures might be associated with breeding or glochidial host attraction.

Premature deaths of test fish may have prevented identification of suitable glochidial hosts. Glochidia grew while attached to flathead catfish. Unfortunately, all but one of these fish died from "Ich" (*Ichthyophtherius multifilis*) before the study was completed. During previous host suitability studies in our laboratory, flathead and channel catfishes exposed to purple wartybach glochidia frequently contract Ich and die prior to excystment of the juveniles. Future host suitability studies should include catfishes among the species to be tested. The channel catfish that facilitated glochidia metamorphosis was collected from the St. Croix River near populations of winged mapleleaf. The importance of resident fishes serving as superior hosts to fishes outside the watershed should be explored more thoroughly.

There is evidence that catfishes serve as glochidial hosts to several *Quadrula* species. Flathead and channel catfish have been shown to be suitable hosts for gulf mapleleaf glochidia (Howells 1997). Wartyback (*Quadrula nodulata*) glochidia naturally infest the gills of channel and flathead catfish (Wilson 1914, Coker *et al*. 1921). Pimpleback (*Quadrula pustulosa*) glochidia are thought to use several Ictalurids as hosts including: black and brown bullheads, channel and flathead catfishes among others (Howard 1912, Howard, 1914, Wilson 1914, Coker *et al*. 1921, Weiss 1993). Mapleleaf (*Quadrula quadrula*) glochidia are thought to utilize flathead catfish as hosts (Howard and Anson 1922, Romano *et al*. 1990, Romano *et al*. 1991). This evidence suggests that Ictalurids are likely natural glochidial hosts for winged mapleleaf.

Acknowledgments

Several people and institutions provided support for this study. We thank Dave Heath, Ron Benjamin, Mark Endris, and Rhonda Kenyon of the Wisconsin Department of Natural Resources for collecting and making observations of gravid mussels, and suggestions that improved the study. Bob Whaley, Jeff Woods, and Byron Karns of the St. Croix National Scenic Riverway, National Park Service were instrumental in facilitating catfish collection from the St. Croix River, and Bob Hay, Wisconsin Department of Natural Resources for assistance in developing ideas discussed in this paper. The USFWS contributed discretionary funding for this project. Funding for this study was also provided by federal aid under Section 6 of the Endangered Species Act of 1973 with matching funds from the Wisconsin Department of Natural Resources. Support for Anne Kapuscinski came in part from the Minnesota Sea Grant College Program, Department of Commerce under NOAA/NA86AA-D-SG112, project R/A-5.

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Figure 1. Swollen excurrent aperture of winged mapleleaf during brooding period.

Figure 2. Winged mapleleaf mussel releasing conglutinates in laboratory.

Figure 3. Conglutinates released by winged mapleleaf.

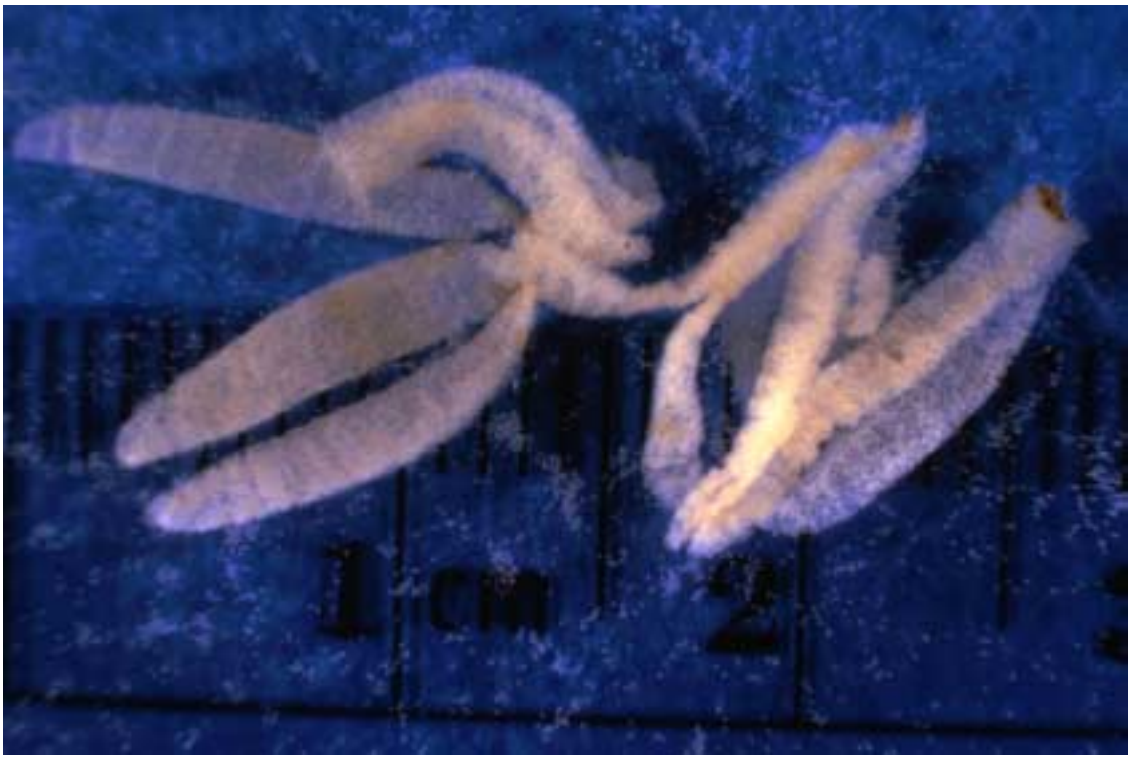

Figure 4. Winged mapleleaf glochidia.

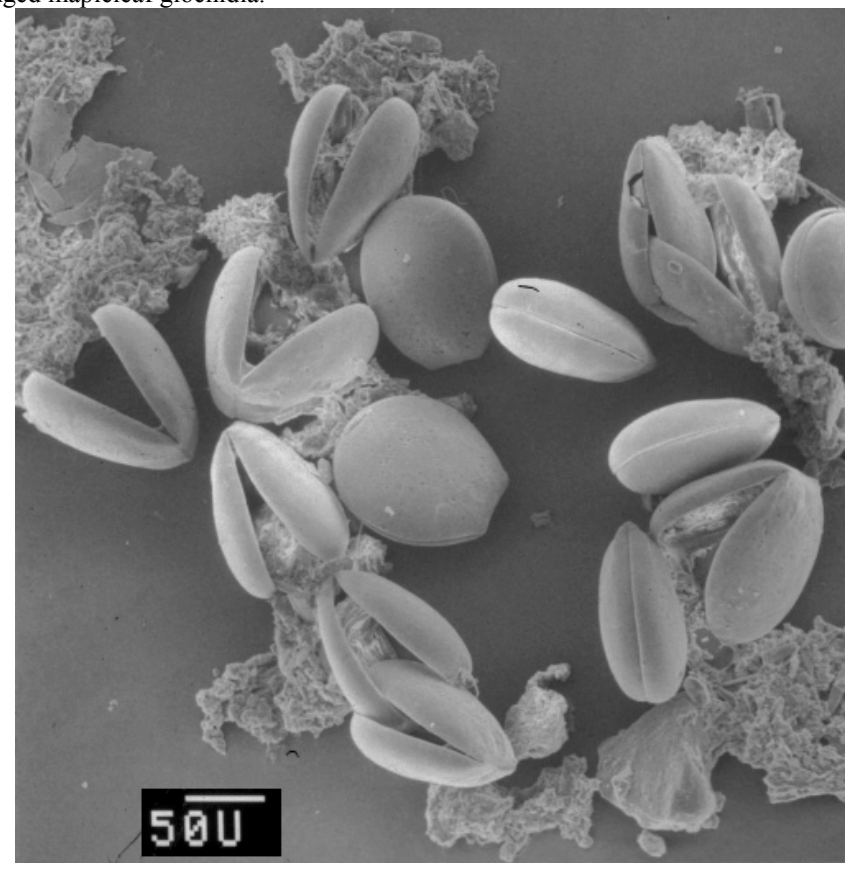

Figure 5. Winged mapleleaf glochidium nearly adducted.

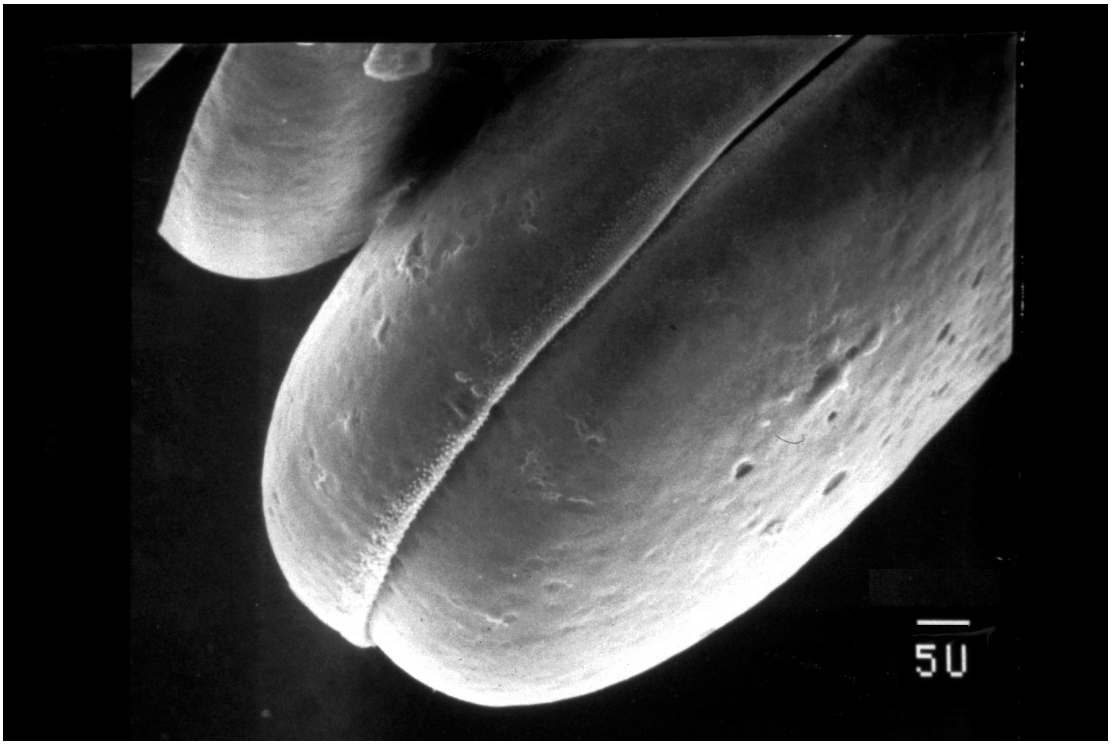

Figure 6. Winged mapleleaf glochidium micropoints.

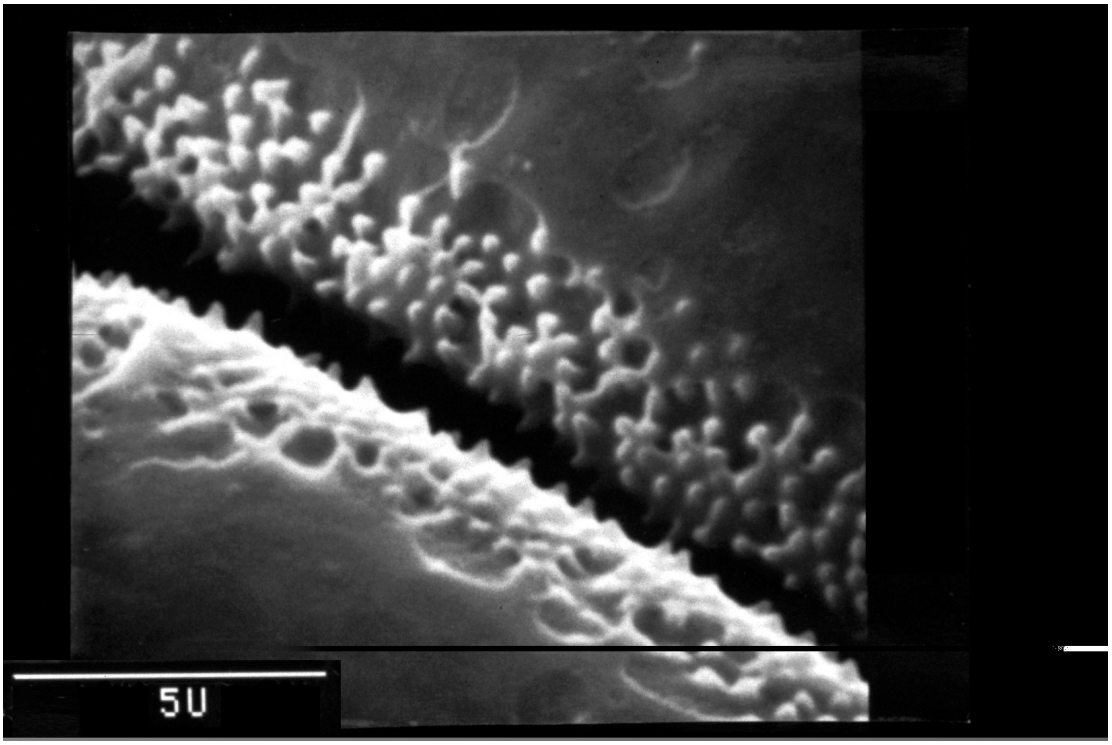

Figure 7. Premetamorphosed winged mapleleaf juvenile nearly double in size.

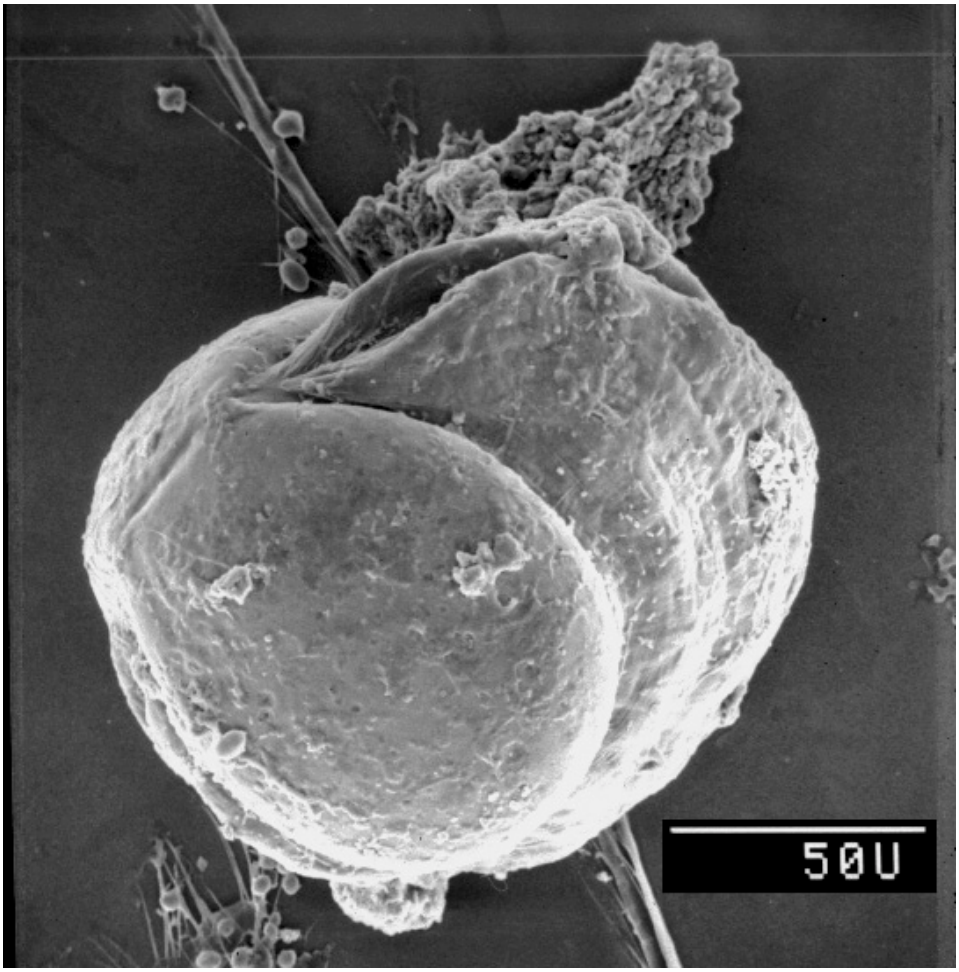

Table 1. Vertebrates that did not facilitate metamorphosis of winged mapleleaf glochidia.

Common name	Number of individuals inoculated	Number of survivors	Glochidia attachment period (days)
1998			
brown bullhead (Trial I)	6	3	27-29
burbot (Trial I)	4	4	5-8
burbot (Trial II)	1	1	2-11
burbot (Trial III)	2	2	1-2
channel catfish (Trial I)	16	1	36-39
channel catfish (Trial II)	36	0*	29-34
common shiner	4	4	1-2
creek chub	4	3	2-5
fathead minnow (Trial I)	1	1	1-2
fathead minnow (Trial II)	26	26	1-3
Johnny darter (Trial I)	38	38	2-5
Johnny darter (Trial II)	9	9	1-3
Johnny darter (Trial III)	2	2	1-2
lake sturgeon	4	4	1-2
lamprey *sp*. (ammocoete)	1	1	unclear
mimic shiner	14	14	1-2
mudpuppy	2	2	1-2
n. hognose sucker	1	1	1-3
northern pike	8	7	2-8
orange-spotted sunfish	2	2	1-2
pumpkinseed	4	4	1-2
rainbow darter	1	1	1-3
river shiner (Trial I)	7	7	1-2
river shiner (Trial II)	1	1	1-3
rock bass	17	17	1-2
sauger	4	4	1-2
shorthead redhorse	22	21	1-3
shovelnose sturgeon	2	2	1-2
slimy sculpin	13	13	2-8
smallmouth bass	12	12	1-2
spotfin shiner (Trial I)	7	7	1-2
spotfin shiner (Trial II)	11	11	1-3
stonecat	1	1	1-11
stoneroller	16	16	8-11
walleye	10	9	1-2
white bass	22	0*	8-11
white sucker	14	14	1-3
yellow perch	17	17	5-8
Number of trials	38		
Number of species tested	30		
1999			
black bullhead	20	18	61-68
Brown bullhead (Trial II)	1	1	Unclear

Flathead catfish	3	1	74-82
Slender madtom	1	1	94-95
Tadpole madtom	4	4	1-4
Yellow bullhead	9	9	108-118
Number of trials	6		
Number of species tested	6		
Total number of trials	44		
Total number of species tested	35		

* - Incomplete trial, test subjects died before completion of the study.

Table 2. Fish that facilitated metamorphosis of winged mapleleaf glochidia.

Common name	Number of individuals inoculated	Number of survivors	Glochidia attachment period (days)	Number of juveniles collected
Channel catfish	1	0*	117-123	2

* - Incomplete trial, test subjects died before completion of the study.

Table 3. Unknown juvenile mussels recovered from St. Croix River fishes.

Common name	Number of individuals	Number of survivors	Number of glochidia sloughed	Number of juveniles excysted
1998				
Channel catfish	1	1	4	0
1999				
lake sturgeon	1	1	19	110

EXAMINATION THE LARVAL STAGE (GLOCHIDIA) OF THE WINGED MAPLELEAF MUSSEL (*Quadrula fragosa*)

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Final Report to National Park Service and Wisconsin DNR

September 27, 1998

ABSTRACT

The goal of this study was to develop a method to identify the glochidia (larval stage) of the endangered winged mapleleaf mussel (*Quadrula fragosa*). This species of mussel is currently found only in the St. Croix River and little is known of its basic biology. Freshwater mussels utilize a fish host for dispersal of the larval stage and the species of fish which are suitable hosts for *Q. fragosa* are unknown.

We explored 2 methods to differentiate the glochidia of *Q. fragosa* from other species of unionids in the St. Croix River. One method involved collecting glochidia from species of *Quadrula* and taking morphometric measurements on specimens under a scanning electron microscope (SEM). The other method involved using DNA fingerprinting using the polymerase chain reaction (PCR). We examined the ITS-1 region of the genome and subjected amplified DNA to a variety of restriction enzymes.

There were differences in the sizes of the glochidia examined for 3 species of *Quadrula*. We obtained glochidia from one Quadrula *fragosa* collected in September 1998. These glochidia were smaller than those obtained from *Q. pustulosa* or *Q. metanevra*. While this method holds promise for identification of *Q. fragosa* glochidia more data is required.

We were able to differentiate *Q. fragosa* from the other 16 species of mussels examined from the St. Croix River. This differentiation could be done by using a single restriction enzyme (MSP1). We were able to obtain sufficient DNA from a small number of glochidia to carry out the PCR reaction. This method provides a simple diagnostic for *Q. fragosa* glochidia.

INTRODUCTION

Winged mapleleaf mussels are listed as endangered under the Federal Endangered Species Act of 1973, as amended, and under Minnesota and Wisconsin State laws. The only known location of the winged mapleleaf mussel is a 20-kilometer (12.5-mile) segment of the Lower St. Croix National Scenic Riverway, located immediately downstream of the

dam at Taylor's Falls, Minnesota, and St. Croix Falls, Wisconsin (Hornbach et. al, 1996). This particular stretch of the Lower St. Croix National Scenic Riverway is administered as a unit of the National Park Service (NPS).

In winged mapleleaf mussels, as in almost all species of the family Unionidae, males release sperm into the surrounding water, which then may be taken in by female mussels (through the incurrent siphon) (McMahon, 1991). Fertilization by the male sperm occurs within the female. The fertilized eggs develop into an early immature form called glochidia. When sufficiently developed, glochidia are released from the female's body into the surrounding water and some may contact and attach to the gills or fins of suitable fish (or amphibians for a very few unionidae). Attaching glochidia encyst in the gill or fin tissue of the host fish, they grow until sufficiently developed to survive on their own, excyst, drop to the bottom of the lake or stream and begin their lives as little mussels. The mussels will survive and mature if they land in a suitable spot, and IF the glochidia originally attached to the required host fish species. Freshwater mussels may use one or several fish species as hosts, depending on mussel species, but suitable fish species, be it one or several species, is obligatory for each mussel species. Glochidia die if they fail to attach to the required species of fish. If the required glochidial host fish species is/are removed or otherwise absent, adult mussels live, but can not reproduce; mussel beds persist a while, but decline and disappear. In summary, for successful development, maturation and reproduction of mussels to occur, a suitable host must be present in sufficient numbers. The survival of all mussel species within the Unionidae family is dependent upon this relationship.

Currently the winged mapleleaf mussel's host fish is unknown. Before 1997, no gravid female winged mapleleaf mussels have been found in the present area of interest; few juvenile winged mapleleaf mussels have been found between 1988 and 1998, a period of intensive scientific study of the winged mapleleaf mussel in the St. Croix River. These two pieces of evidence suggest the present winged mapleleaf mussel population may not be maintaining itself, that it will decline and vanish as its individual mussels age and die without reproducing.

The winged mapleleaf mussel is a Recovery Priority 2C *species* under the

U.S. Fish and Wildlife Service (USFWS) recovery priority system for all listed plants and animals. Recovery Priority 2 is the second highest priority and is applied only to species of polytypic genera whose danger of extinction or catastrophic decline is both great and imminent. The only higher priority for action is for similarly imperiled species in monotypic (single-species) genera. ("C" denotes potential conflicting interests over recovery of the species.)

The USFWS engages species experts and other experts to prepare recovery plans for threatened and endangered species. These plans describe the threats and problems facing the species and contain specific *tasks* to eliminate the threats and to overcome the problems so the species can be removed from the Federal threatened and endangered species list. The tasks are prioritized in a system applied to recovery plans for all species. Priority 1 tasks, the highest priority, are actions "...that must be taken to prevent extinction or to prevent the species from declining irreversibly in the foreseeable future."

The *Winged Mapleleaf Mussel Recovery Plan* (USFWS, 1997) identifies glochidial fish host identification as a Priority 1 task; the Winged Mapleleaf Mussel Recovery Team very recently identified glochidial host fish identification as its top priority project for USFWS FY-1997 endangered species funding. The USFWS, the NPS, the Minnesota and Wisconsin Departments of Natural Resources (MN and WI DNRs), and academic species experts recognize the critical need for prompt host fish identification. Despite the recognized and widely supported need, funding for the work is problematic because of endangered species budget limitations.

DESCRIPTION OF CURRENT PROJECT:

This project seeks, as part of a larger research project being conducted jointly with the WI DNR and the University of Minnesota, to determine the host fish of the winged mapleleaf mussel. The result of the proposed work would be identification of the fish species that serve as glochidial hosts to the winged mapleleaf mussel. The specific product for this

contract is a visual and a DNA-based glochidial taxonomic key for members of the genus *Quadrula* , the subfamily containing winged

mapleleaf mussels. The key would contain only *Quadrula* species occurring in the present range of the winged mapleleaf mussel, *i.e*., the St. Croix River. Funding for this project has come from both the WI-DNR and the National Park Service, St. Croix Scenic Riverway. In addition, support has been provided by Macalester College and by a Howard Hughes Medical Institute Grant to Macalester College.

METHODS:

The methods of the Polymerase Chain Reaction (PCR) portion of the project followed closely those established White (1994) and White *et al.* (1994). DNA was extracted from foot tissue of adult specimens for all species examined except for *Quadrula fragosa*, whose DNA was extracted from a whole glochidium provided by Mark Hove of the University of Minnesota. During DNA extraction, 5-50 mg of tissue was excised from each sample and minced with sterile scalpel blades on chilled glass microscope slides. For *Q. fragosa* we were able to extract sufficient DNA from a small group of glochidia (5-10) to carry out the PCR analysis. Minced tissue was homogenized and incubated for two hours in a standard extraction buffer. Nucleic acids were isolated in a series of Phenol-Chloroform extractions, crystallized in chilled ethanol, and dried. The dried, crystallized DNA was resuspended in water and analyzed spectrophotometrically to determine purity and concentration.

The ITS-1 region of rDNA was amplified via PCR. This region presented inter-specific variation in the mussel taxa of White *et al* and has been shown to have an intermediate rate of sequence evolution (Mindell and Honeycutt, 1990). Amplified DNA was digested by five restriction endonucleases: Msp I, Mse I, Sau 3a I, Hae III, and Hin P1. The digested DNA was applied to agarose gels and separated by electrophoresis. These gels were then stained with ethidium bromide and visualized with UV light. Approximate length (in base pairs) of digested DNA fragments were established by comparing migration distance on the gels with standard molecular weight ladders on either side of the gels. DNA of each species was digested by five different restriction endonucleases in an attempt to reveal a unique pattern of DNA fragments—a genetic “fingerprint”--for each species examined. To

date, we have extracted DNA from 27 of the 40 St. Croix River mussels and have genetically analyzed 17 of these species.

Studies by Waller et al (1988) have shown that taking measurements of the glochidial shells of mussels and then performing a statistical analysis can allow for the identification of glochidia to species. We obtained glochidia from 3 species of *Quadrula* from Mark Hove at the University of Minnesota for examination under the scanning electron microscope (SEM): *Quadrula pustulosa, Quadrula metanevra,* and *Quadrula fragosa*. The glochidia from these samples were stored in 100% ethanol at room temperature. A Pasteur pipette was used to transfer glochidia to the SEM puck. The puck had previously be coated with *poly-L-lysine* and silver-conducting paint. Once transferred to the puck, the 100% ethanol was allowed to evaporate off the glochidia under a hood. With the sample dry, the puck was placed in a gold sputter-coating apparatus and coated for 2 minutes at 40 mA.

The samples were then examined under the Zeiss DSM 960 A scanning electron microscope and pictures were digitally captured via an Oxford Link ISIS device. Due to the varied orientation of glochidia on the puck, different angles could be captured and measured. Twenty seven individual glochidia from 1 adult *Q. fragosa,* 2 adult *Q. pustulosa* and 2 adult *Q. metanevra* females were examined. Measurements were taken of length (maximum dorso-ventral dimension), width (maximum anterior-posterior dimension), and hinge length. Magnifications used in specimen examination ranged from 200 to 500X.

RESULTS AND DISCUSSION

As part of this project, Mark Hove of the University of Minnesota and David Heath of the WI DNR conducted field work to collect glochidia from gravid mussels in the St. Croix River. Much to the surprise of these researchers the did not find any gravid *Quadrula fragosa* in the late spring or summer of 1997 or 1998, despite intensive sampling. Most *Quadrula* species are short-term brooders, releasing their glochidia in spring or summer. These researchers did however recovery one gravid *Quadrula fragosa* in the fall of 1997 and several in the fall of 1998.

Genetic Analysis

We genetically analyzed 17 species of the St. Croix River mussels using the DNA fragment patterns of five restriction endonucleases (Appendix B). We were able to distinguish the endangered *Quadrula fragosa* from the other 3 members of the genus *Quadrula* present in the St. Croix River using fragment patterns of Msp I (Figure 1 and Appendix C). *Q. fragosa* was

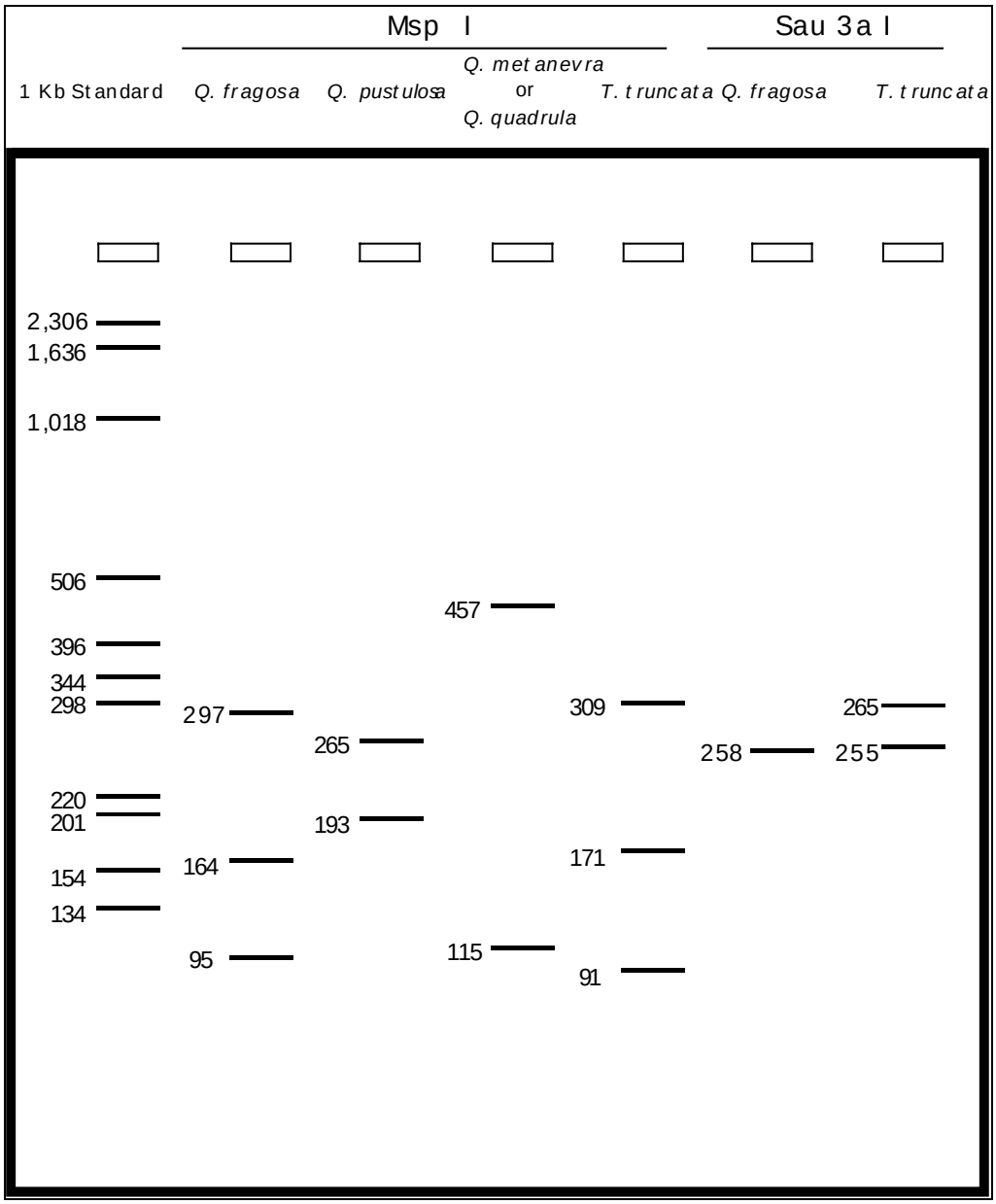


Figure 7 - Reconstruction of a PCR gel showing unique banding patterns.

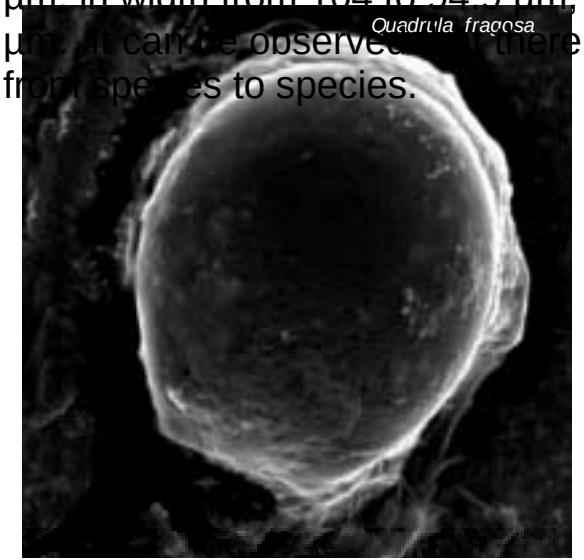
further distinguished from the other 16 species that we examined with the fragment pattern from Sau 3a I (Figure 1 and Appendix C). We were able to distinguish all 17 mussels examined from one another, but those results are beyond the scope of this report. It should be noted, however, that many of the DNA fragment patterns that we obtained were identical to those given by White (1994) and White *et al* (1994), indicating that Restriction Fragment Length Polymorphism analysis is robust across great geographic areas (White's work was conducted in Pennsylvania).

In essence, to test whether a glochidium removed from a fish were a member of the endangered *Quadrula fragosa* one would use the following protocol:

1. Extract and amplify DNA from glochidium.
2. Digest DNA with Msp I
3. If fragments match Msp I pattern for Q*. fragosa,* digest DNA with Sau 3a I (figure 1).
4. If fragments match *Q. fragosa* pattern for Sau 3a I, DNA is from Q*. fragosa.* If DNA does not match this pattern, DNA is from *Truncilla truncata.*

Scanning Electron Microscopy

The morphology of glochidia are quite variable and work from the early 1900s (e.g. Surber, 1915) and more recently (e.g. Hoggarth, 1988) have shown that there are detectable differences at both the family and genera level. The differentiation at the species level, however often requires examining small morphological features (such as hooks, spines and tubercles) or by examining subtle differences in size or shape (Waller et al. 1988). Clear morphological differences between species can be observed from the SEM images (Fig 2-4). *Quadrula fragosa* and *Quadrula metanevra* appear similar in shape, while the narrow hinge of *Quadrula pustulosa* makes it easily distinguishable. Measurements (to a tenth of a micrometer) were taken using the Link ISIS device attached to the SEM. These measurements are attached (Appendix A).*Q. pustulosa* varied in length from 359 to 310 µm, in width from 290 to 251 µm, and in hinge length from 136 to 105 µm. *Q. metanevra* varied in length from 259 to 242 µm, in width from 232 to 213 µm, and in hinge length from 110 to 102 µm. *Q. fragosa* varied in length from 122 to 110 µm, in width from 104 to 94.9 µm, and in hinge length from 47.8 to 43.3 µm. It can be observed that there was a large variance in overall size from species to species.



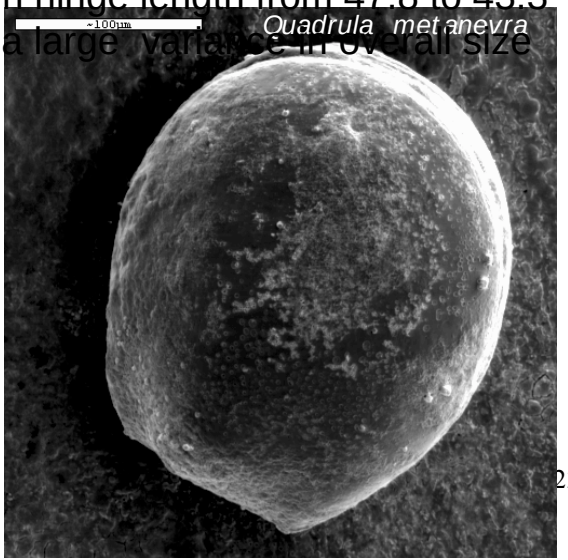
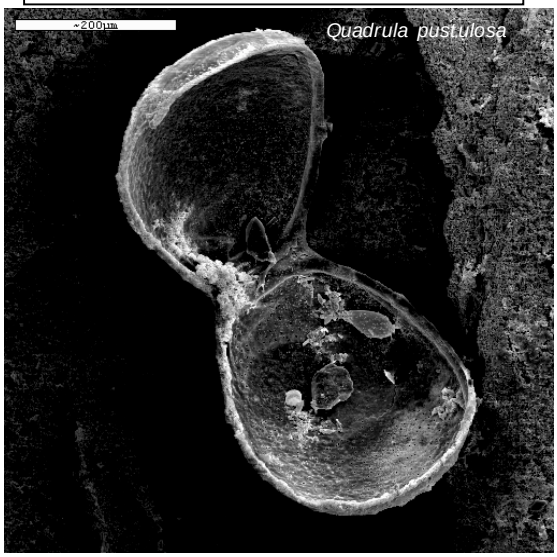


Figure 2 – SEM of *Quadrula fragosa.*

Analyses of variance indicate that there are significant difference in the length, width and hinge length among species (Fig. 5). Hoggarth (1988) provides a length:width ratio of 1.33 for *Q. pustulosa* compared to the

Figure 4 - SEM of Quadrula pustulosa.

- SEM of *Quadrula metanevra.*



mean value of 1.25 (sd=0.10) from this study.

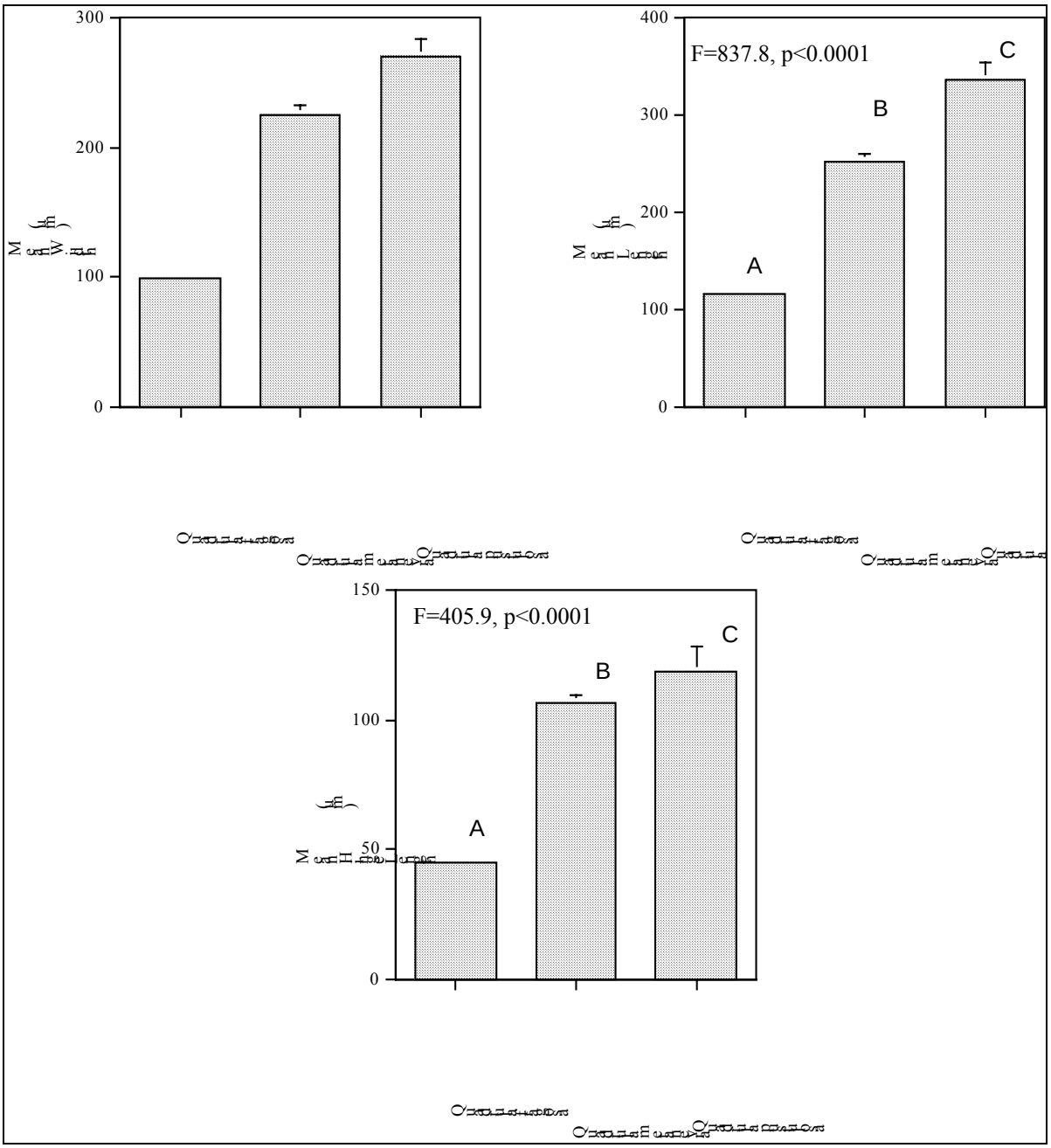


Figure 5 - Mean width, length and hinge length of 3 species of Quadrula. Bar with different letters are significantly different.

Since glochidia were taken from only 1 *Q. fragosa* it is possible that we are examining individual variation rather than variation among species. For example it is possible that the glochidia were not mature (although Mark Hove has used these glochidia to infect fish hosts). One way to

overcome the differences in absolute size that may be due to differences in maturity is to examine ratios of these measurements. Ratios of hinge length versus width and hinge length verses glochidium length were computed. Analyses of variance are provided in Figure 6. It is clear that *Q. pustulosa and Q. metanevra* can be differentiated based on the difference in these ratios but that it is not possible, with this small sample size to differentiate between *Q. fragosa* an other species of *Quadrula.*

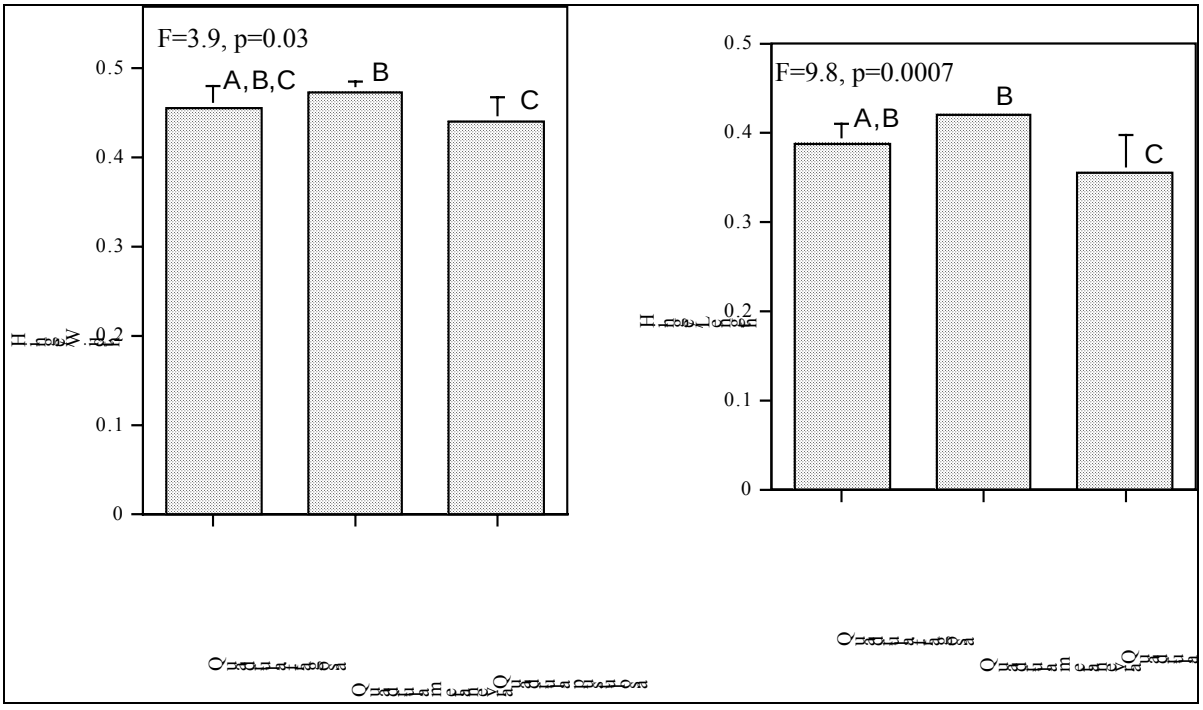


Figure 6 - Mean Hinge/width and hinge/length ratios for 3 species of *Quadrula*. Bars with different letters are significantly different.

It appears then that *Q. fragosa* are significantly smaller than those of other *Quadrula* species in the St. Croix, but are similar in shape to *Q. metanevra.* The small *Q. fragosa* glochidia found are within the size range found for other members of the Ambleminae and Lampsilinae (Hoggarth, 1988) and thus these glochidia need to be compared with glochidia from other species of unionids.

CONCLUSION

While both morphological examination and PCR analysis provide means to identify the glochidia of *Q. fragosa* , it appears that using PCR is the

most effective means of providing for identification.

ACKNOWLEDGEMENTS

We acknowledge funding provided by the National Park Service and the Wisconsin DNR. Also student support came from the Howard Hughes Medical Institute and the Keck Foundation through grants to Macalester College. Macalester College also provided summer housing for students and administrative support. Dr. James Straka played a major role in overseeing the genetic analysis. Former students Zachary Hayden and Pete Doel also contributed to early portions of the genetic analysis. Dr. Russell Whitehead provided expertise for the SEM portion of this work.

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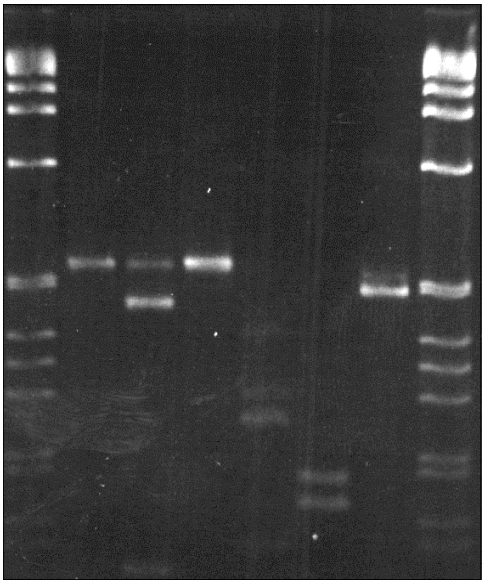
Appendix A. Measurements taken from *Quadrula* glochidia.

Species	Sample	Width (μm)	Length (μm)	Hinge Length (μm)	Hinge: Width Ratio	Hinge: Length Ratio
Quadrula metanevra	A1	227	251	105	0.46	0.42
Quadrula metanevra	A2	222	254	109	0.49	0.43
Quadrula metanevra	A3	232	258	106	0.46	0.41
Quadrula metanevra	A4	213	242	102	0.48	0.42
Quadrula metanevra	A5	229	254	107	0.47	0.42
Quadrula metanevra	A6	229	259	110	0.48	0.42
Quadrula pustulosa	A1	272	312	115	0.42	0.37
Quadrula pustulosa	A2	273	357	118	0.43	0.33
Quadrula pustulosa	A3	289	312	129	0.45	0.41
Quadrula pustulosa	A4	274	323	130	0.47	0.4
Quadrula pustulosa	A5	290	359	123	0.42	0.34
Quadrula pustulosa	A6	277	310	136	0.49	0.44
Quadrula pustulosa	B1	255	346	115	0.45	0.33
Quadrula pustulosa	B2	280	344	110	0.39	0.32
Quadrula pustulosa	B3	251	345	105	0.42	0.3
Quadrula pustulosa	B4	265	330	112	0.42	0.34
Quadrula pustulosa	B5	258	356	119	0.46	0.33
Quadrula pustulosa	B6	256	340	114	0.45	0.34
Quadrula fragosa	A1	98.4	117	46.6	0.47	0.4
Quadrula fragosa	A2	94.9	110	47.8	0.5	0.43
Quadrula fragosa	A3	97.4	115	44.9	0.46	0.39
Quadrula fragosa	A4	102	113	43.7	0.43	0.39
Quadrula fragosa	A5	99.8	118	44.5	0.45	0.38
Quadrula fragosa	A6	95.5	116	43.3	0.45	0.37
Quadrula fragosa	A7	100	118	43.5	0.43	0.37
Quadrula fragosa	A8	101	122	45.2	0.45	0.37
Quadrula fragosa	A9	100	116	47.7	0.48	0.41
Quadrula fragosa	A10	104	119	44.7	0.43	0.38

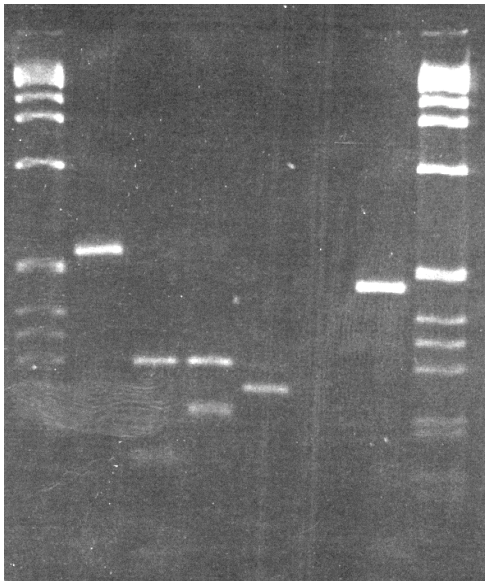
Appendix B. List of species tested with PCR

Actinonaias ligamentina
Amblema plicata
Ellipsaria lineolata
Elliptio dilatata
Fusconaia flava
Lampsilis cardium
Lampsilis siliquoidea
Obliquaria reflexa
Obovaria olivaria
Pleurobma coccineum
Potamilus alatus
Quadrula fragosa
Quadrula metanevra
Quadrula pustulosa
Quadrula quadrula
Strophitus undulatus
Truncilla truncata



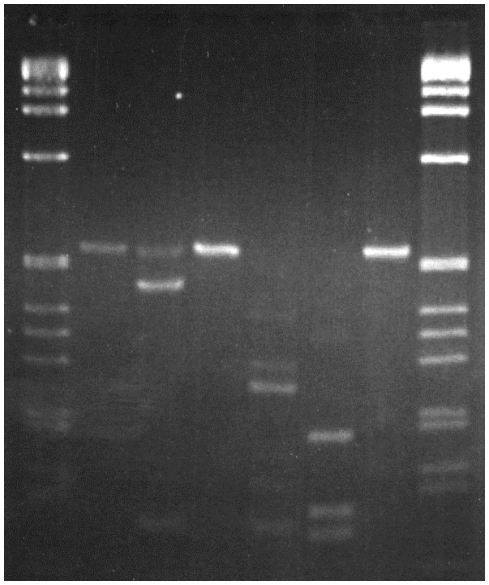
Quadrula fragosa

1-ladder
2-uncut
3-Msp I
4-Mse I
5-Sau 3a I
6-Hae III
7-Hin P1
8-ladder



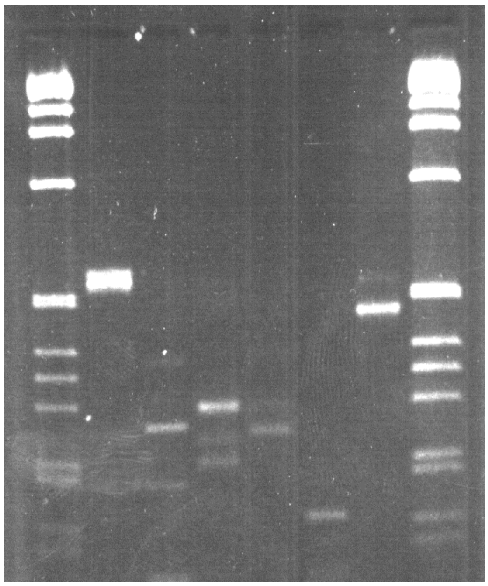
Quadrula pustulosa

1-ladder
2-uncut
3-Msp I
4-Mse I
5-Sau 3a I
6-Hae III
7-Hin P1
8-ladder



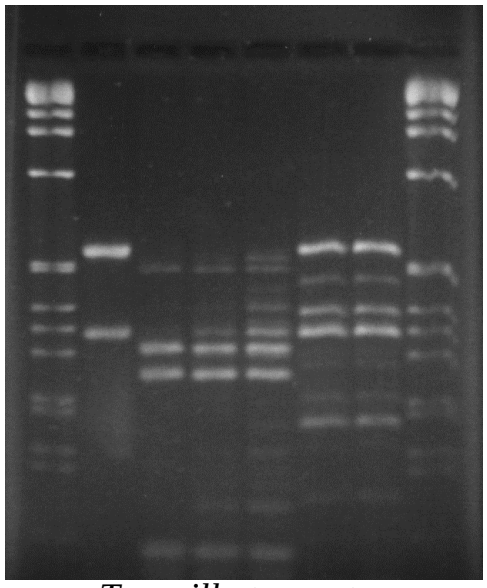
Quadrula metanevra

1-ladder
2-uncut
3-Msp I
4-Mse I
5-Sau 3a I
6-Hae III
7-Hin P1
8-ladder



Quadrula quadrula

1-ladder
2-uncut
3-Msp I
4-Mse I
5-Sau 3a I
6-Hae III
7-Hin P1
8-ladder



Truncilla truncata

1-ladder
2-uncut
3-Sau 3a I
4-Sau 3a I
5-Sau 3a I
6-Hae III
7-Hae III
8-ladder